



# ABSTRACTBOOK

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**20<sup>th</sup> Symposium on  
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**ÖGHMP**  
Österreichische Gesellschaft  
für Hygiene, Mikrobiologie und  
Präventivmedizin



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# **ORAL PRESENTATIONS**

## **SESSION 1 – METAGENOME, MICROBIOME AND COMMUNITY ANALYSIS**

### **01 Quantitative Viral Metagenomic Method Combining Long and Short Read Sequencing**

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Metagenomics are allowing unprecedented insight into microbial communities. Next generation sequencing (NGS) has led to many discoveries about viruses, but three notable challenges exist in applying NGS to study the viromes of complex matrices. Despite being the most highly abundant members of most communities, viral nucleic acids are a small portion of the total nucleic acids. Efficient viral enrichment methods are needed. Viral isolation and enrichment methods are not equally efficient. Optimized methods are needed to quantitatively compare viral representation across samples. Viral genomes are mosaic with a high mutation rate and, therefore, difficult to assemble with short reads produced with Illumina sequencing technologies. Supplemental sequencing technologies need to be explored.

To address these challenges, our research advances NGS methods with a quantitative sequencing approach on the isolated virus fraction from wastewater with a combination of long and short read sequencing. Wastewater viruses are concentrated with tangential and dead-end ultrafiltration 575-fold in influent and 1,150-fold in effluent. The viruses are purified with chloroform to lyse cells and DNase to degrade extra-viral DNA. The concentration and purification protocol recovered approximately half of the viral DNA while removing 4-logs of 16S rRNA.

For quantitative comparisons, 86 dsDNA and 5 ssDNA internal standards were spiked into samples at 1% of the total DNA concentration prior to simultaneously sequencing ssDNA and dsDNA on Illumina NovaSeq. The internal standards were used to determine sequencing efficiency with respect to GC content. The Illumina sequencing of the dsDNA phages was complemented by long-read sequencing on the Oxford Nanopore GridION. Short and long read sequencing data was *de novo*-assembled to identify phages in influent and effluent. The absolute abundance of contigs was determined by mapping the short reads to the contigs and converting the number of reads aligning to contigs to a concentration using the sequencing efficiency.

### **02 Factors shaping the intestinal microbiome of vertebrate faecal sources: unravelling the role of diet and host phylogeny**

Georg H. Reischer (A, B), Nicholas D. Youngblut (C), William Walters (C), Nathalie Schuster (A), Chris Walzer (D), Gabrielle Stalder (D), Ruth E. Ley (C), Andreas H. Farnleitner (A, B, E)

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High-throughput sequencing is allowing unprecedented insight into microbial community structure. Understanding the composition and dynamics of intestinal microbiota is an essential basis for assessment of faecal pollution in the environment and for the development of molecular diagnostic tools. According to previous studies community assembly in the vertebrate gut seems to be modulated by multiple factors such as diet or host phylogeny though there is disagreement as to the relative contribution of these drivers. To resolve this, we compiled and analysed a large and highly diverse animal 16S rRNA faecal microbiome dataset, which in contrast to previous studies, comprises 80 % wild animals. It included data from 213 samples from 128 different species of Mammalia, Aves, Reptilia, Amphibia, and Actinopterygii. Using bioinformatic tools we managed to decouple the effects of host evolutionary history and diet on gut microbiome diversity and could show that each factor

modulates different aspects of diversity. Moreover, we found particular microbial taxa associated with host phylogeny and, to a lesser extent, with diet. Interestingly, the signal of cophylogeny was much stronger in Mammalia compared to non-mammalian hosts. We hypothesize that this difference might be due to the more sophisticated digestive physiology of mammals, especially in the group of the fore-gut fermenting ruminants. Another factor might be more pronounced vertical transmission of microbiota between generations in mammals during birth and nursing. In the context of faecal pollution characterisation our findings suggest that there is a gradient from low to high host-association of intestinal populations from fish and amphibians to reptiles and birds to mammals showing the highest level of coevolution. Taken together with the strong influence of diet on abundance patterns and the presence of transient and opportunistic populations in the investigated microbiota this study highlights future challenges and opportunities in molecular faecal pollution detection and source tracking.

### **03 Impacts of anthropogenic activities on the health related microbes in a river ecosystem**

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Anthropogenic activities (e.g. wastewater discharge and pesticide use) have a great impact on the biotic properties of natural aquatic ecosystems, especially the microbial diversity and function. A number of reports have documented the impacts of anthropogenic activities on the variations in the microbial community, but directly using microbial community indices to track the pollution source and discern the levels of anthropogenic activities is still limited. Here, we integrated flow cytometry, metagenomic sequencing and chemical analyses to investigate the composition, and function of the health related microbes (antibiotic resistant bacteria and pathogen) in three areas along a gradient of anthropogenic disturbance (less-disturbed mountain area, wastewater-discharged urban area, and pesticide-using agricultural area) in a river ecosystem. Multiple statistical methods were used to explore the causal relationships between the changes in environmental factors and the health related microbial variation. Results showed that anthropogenic activities (wastewater discharge and agricultural fertilizer) facilitated the production of potentially harmful bacteria, affected the distribution of dominant pathogen species, and accelerated the horizontal gene transfer of antibiotic resistant genes (ARGs). ARGs abundance persistently increased from the upstream (mountain area) to midstream (urban area), and to downstream (agriculture area). By co-occurrence network analysis and source track technique, wastewater treatment plant was identified as the main sources of ARGs. *Pseudomonas*, identified as the most abundant host of ARGs, changed obviously among three areas with significantly higher aminoglycoside, beta-lactam, sulfonamide and quinolone in wastewater-discharged urban area. Finally, we also identified the types and abundance of pathogens carrying the ARGs, which differently distributed in three areas.

### **04 Assessing the spatial and temporal variability of bacterial communities in two Bardenpho wastewater treatment systems via Illumina MiSeq sequencing**

Samendra Sherchan, Jia Xue, Bradley Schmitz, Ian Pepper, Charles Gerba

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Wastewater treatment plants (WWTPs) serve an important function by reducing organic loads in wastewater before discharge into the environment. Such facilities treat influent from a mixture of sources containing household sewage, storm water runoff, industrial effluent, and reclaimed water. Next generation sequencing provides new insights into the diversity and ecophysiology of bacteria communities throughout wastewater treatment plants (WWTP), as well as the fate of pathogens in wastewater treatment system. In the present study, we investigated the bacterial communities and human-associated Bacteroidales (HF183) marker in two WWTPs in North America that utilize Bardenpho treatment processes. Although, most pathogens were eliminated during wastewater

treatment, some pathogenic bacteria were still observed in final effluents. The HF183 genetic marker demonstrated significant reductions between influent and post-Bardenpho treated samples in each WWTP, which coincided with changes in bacteria relative abundances and community compositions. Consistent with previous studies, the major phyla in wastewater samples were predominantly comprised by Proteobacteria (with Gammaproteobacteria and Alphaproteobacteria among the top two classes), Actinobacteria, Bacteroidetes, and Firmicutes. Dominant genera were often members of Proteobacteria and Firmicutes, including several pathogens of public health concern, such as *Pseudomonas*, *Serratia*, *Streptococcus*, *Mycobacterium* and *Arcobacter*. Pearson correlations were calculated to observe the seasonal variation of relative abundances of gene sequences at different levels based on the monthly average temperature. These findings profile how changes in bacterial communities can function as a robust method for monitoring wastewater treatment quality and performance for public and environmental health purposes.

## **05           The Burden and Direct Healthcare Cost of Waterborne Disease in the United States: Implications for Public Health and Environmental Scientists**

Sarah A. Collier, Li Deng, Katharine M. Benedict, Kathleen E. Fullerton, Jonathan S. Yoder, Vincent Hill and Michael J. Beach

Centers for Disease Control and Prevention (U.S.)

**Background:** Despite advances in the treatment of water in the United States, waterborne disease and outbreaks continue and are associated with a variety of water sources (drinking, recreational, environmental) and exposure routes (ingestion, contact, inhalation). CDC has completed an estimate of the burden of waterborne disease in the United States, measured through numbers of illnesses, emergency department (ED) visits, hospitalizations, deaths, and associated costs, to help direct prevention activities and set public health goals.

**Methods:** We chose 17 waterborne diseases for which surveillance data, billing data, or literature estimates indicated domestic waterborne transmission was plausible, substantial burden of illness or death was likely, and data were available. Adapting previously developed methods, we used a series of disease-specific multipliers to adjust the reported/document number of cases of each disease for under-reporting, under-diagnosis, proportion domestically acquired, and proportion transmitted via water to generate point estimates with 95% credible intervals (CrI). We estimated the number of illnesses, ED visits, hospitalizations, and deaths, and the costs of ED visits and hospitalizations due to waterborne disease transmitted in the United States in 2014.

**Results:** An estimated 7.2 million waterborne illnesses (CrI 3.9–12.0 million) occur annually, including 601,681 (CrI 365,061–866,093) ED visits, 117,342 (CrI 85,805–150,070) hospitalizations, and 6,559 (CrI 4,373–8,932) deaths, incurring US\$3.2 billion (2014 dollars) in direct healthcare costs. Otitis externa (e.g., "Swimmer's Ear") and norovirus infection were the most common illnesses. Hospitalizations and deaths were predominantly caused by environmental pathogens that are commonly associated with biofilm in plumbing systems (e.g., Nontuberculous mycobacteria, *Pseudomonas*, *Legionella*) costing US\$2 billion annually.

**Conclusions:** Millions of domestically-acquired waterborne illnesses from these 17 infections occur in the United States each year, and incur billions of dollars in healthcare costs. This analysis highlights the roles of enteric and environmental pathogens in waterborne disease in the United States.

**06           The emerging importance of water in produce-associated outbreaks: Lessons from the 2018 United States romaine-lettuce associated *E. coli* O157:H7 outbreaks**

Mia Catharine Mattioli, Amy Kahler, Jen Murphy, Matt Wise, Kevin Gerrity, Stic Harris, Vince Hill  
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*Background:* Between 2009 and 2017, the United States Food and Drug Administration and Centers for Disease Control and Prevention identified 28 foodborne outbreaks of Shiga-toxin producing *Escherichia coli* (STEC) in the U.S. with a confirmed or suspected link to leafy greens. In response to an environmental investigation implicating water in a 2006 multi-state STEC outbreak caused by bagged spinach, regulation was proposed for monitoring agricultural water microbial quality for growing fresh produce. Despite these events, there was a lack of data showing a direct link between the microbial quality of agricultural water and consumer health. In 2018, the U.S. experienced two large STEC outbreaks associated with romaine lettuce. Produce traceback for both outbreaks led to multiple distributors and farms within large growing regions, suggesting agricultural water as a possible contamination source.

*Methods:* To evaluate agricultural water as the source of lettuce contamination, samples of water, sediment, and canal surfaces were collected from irrigation water sources associated with fields linked to the outbreak. Water was collected using large-volume ultrafiltration to facilitate low-concentration pathogen detection, and a species-specific culture technique was used to isolate pathogens from the background community.

*Results/Conclusions:* In both investigations, the outbreak strain of *E. coli* O157:H7 was isolated from the water sources used to grow romaine lettuce, and environmental isolates matched outbreak clinical isolates by pulse-field gel electrophoresis and whole genome sequencing. Despite the commonalities between the outbreaks, agricultural water source and use practices in the outbreak growing regions were vastly different, demonstrating that U.S. surface waters vulnerable to zoonotic pathogens, and the use of this water to grow fresh produce can have direct consumer health impacts. This emerging understanding is leading U.S. food safety stakeholders to evaluate new practices and prevention strategies to improve the safety of water sources for fresh produce production.

**07           Impact of Water, Sanitation, and Hygiene (WASH) interventions on the bacterial pathogen load in households in rural Nepal**

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Water, Sanitation, and Hygiene (WASH) interventions are known to be most effective to reduce severity and prevalence of diarrheal diseases, especially in rural communities. However, monitoring the impact of intervention activities mostly rely on self-reporting surveys. We employed a microfluidic approach to quantify bacterial pathogen in water and surroundings in households to investigate the effectiveness of WASH intervention on the reduction of pathogen load. Two communities with similar socio-economic background and hydroclimatic condition, but one (V1) going through extensive WASH intervention and one (V2) where no intervention activities being conducted (control village) were chosen for the study. Water and sanitation samples were collected from both communities in the summer of 2017 and 2018, i.e., before and after implementation of several activities in V1. A total of 720 samples were taken from these communities which included drinking water, cleaning water, hand wash water samples and swab samples from toilet handles, utensils and water vessels. All samples were subjected to DNA extraction. Twenty pathogens were selected, assays were designed and quantification was done using microfluidic quantitative polymerase chain reaction (mfqPCR). Overall results indicated more pathogen detection in higher concentration in both intervention and control village in 2018. *Enterococcus* spp., *E. coli*, *Legionella pneumophila* was significantly higher ( $p <$

0.01) in swab samples in intervention village post intervention but no such trend was seen in control village. However, *Salmonella typhimurium* ( $p < 0.001$ ) was significantly higher in both intervention and control village in 2018 after implementation of intervention activities. It indicates the inability of current WASH intervention practices to successfully reduce pathogens from households. This study provides a foundation for microbial research of WASH intervention and potentially help with better planning of intervention activities in the future.

## **SESSION 2 – WATER TREATMENT AND DISINFECTION**

### **08 Water Treatment Processes for Preventing Transmission of Schistosomiasis**

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Schistosomiasis is a water-borne neglected tropical disease (NTD) caused by parasitic worms. Infection occurs through contact with cercaria-infested freshwater. Cercariae are shed by freshwater snails and penetrate the human host's skin, leading to schistosomiasis infection. While chemotherapy with praziquantel can have immediate beneficial effects, reinfection can occur rapidly if people are re-exposed to contaminated water. As schistosomiasis control targets become more ambitious and we move towards elimination, interest is increasing in the potentially complementary roles of water, sanitation, and hygiene (WASH) interventions. These may disrupt transmission of the parasite, thereby reducing the likelihood of reinfection following treatment. Water treatment for schistosomiasis control seeks to eliminate viable cercariae from water but the information available to-date about the effectiveness of water treatment processes against cercariae is limited and incomplete. As part of the WISER: Water Infrastructure for Schistosomiasis Endemic Regions project ([www.wiserschisto.com](http://www.wiserschisto.com)), experiments were conducted in the UK, Ethiopia and Tanzania to test the effectiveness of chlorination, sand filtration, and ultraviolet disinfection against schistosome cercariae. *Schistosoma mansoni* cercariae cultured in the Natural History Museum, London were used as well as cercariae and water samples collected from the environment. The effectiveness of each of the treatment processes was examined individually and guidelines have been produced to enable the design of household and community scale water treatment infrastructure that will help minimise re-exposure to unsafe water by providing safe facilities for water contact activities. This work is very timely considering the recent publication of the World Health Organization's toolkit for WASH and NTD programmes, as these two communities come together to find lasting solutions for combating schistosomiasis.

### **09 Development of Sustainable Water Infrastructure for Schistosomiasis Control in Ethiopia**

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Schistosomiasis is among neglected tropical parasitic diseases caused by several species of the genus *Schistosoma* worms affecting over 200 million people in 78 countries worldwide with 90% of the burden currently concentrated in Africa. Many people in developing countries including Ethiopia do not have access to piped water. The repeated contact with cercaria infested water hence increase the risk of contamination in different areas of the country. Slow sand filtration is considered as one of sustainable options for rural water treatment because it is relatively low cost and simple to operate. This study aims to establishing specific design and operational parameters for the use of slow sand filter to eliminate cercaria from water. The filter setup consists of PVC pipes having internal diameter of 15.4 cm with endcap, plastic hose with diameter of 6 mm for water outlet, plastic diffuser and locally available river sand. The parameters chosen for optimization are grain size and depth of fine sand. Hence, three filters constructed were filled with 5 cm from bottom of the filter with gravel size of  $>11.2$  mm , then 5cm of 6-11.2 mm and then 5 cm of 2-6 mm. Then each filter is filled with 60 cm

of mixed grain size sand 0.15-0.85 mm (filter 1), 0.425-0.6 mm sand (filter 2) and 0.15-0.425 mm sand (filter 3). Infected snails were collected from nearby endemic areas (Lake Ziway, Ethiopia) and kept in the lab to produce enough cercariae. Results obtained so far show that filters with sand depth of 60 cm and 70 cm, reduced the number of cercariae more than 95 %. As filter depth increases, the efficiency of cercariae removal also increased. Therefore, we conclude that providing treated with slow sand filter for various uses for rural communities can be a feasible option to control schistosomiasis.

## **10 Inactivation of adenovirus in water by natural and synthetic compounds**

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Millions of people use contaminated water sources for direct consumption, mostly in low- and middle-income countries, representing a public health concern. Chlorine is the most widely disinfection product but can produce toxic by-products, such as trihalomethanes. In this context, natural and synthetic compounds can be an alternative to water disinfection. N-chlorotaurine (NCT), Bromamine-T (BAT) and Grape Seed Extract (GSE) are compounds with outstanding microbicidal activity, as well as high tolerability, low toxicity in human cells and anti-inflammatory or anti-oxidative activity. Therefore, the aim of this study was to assess the inactivation of human adenovirus by NCT, BAT and GSE in water. Distilled water artificially contaminated with recombinant human adenovirus type 5 (rAdV-GFP) was treated with different concentrations of each compound for up to 120 min and viral infectivity was assessed by fluorescence microscopy. The decrease of activity of the compounds in the presence of organic matter was evaluated in water supplemented with peptone. As results, NCT and GSE inactivated approximately 2.5 log<sub>10</sub> of adenovirus after 120 min. With BAT, more than 4.0 log<sub>10</sub> decrease was observed within 10 min. The oxidative activity of 1% BAT decreased by 50% in 0.5% peptone within a few minutes, while the reduction was only 30% for 1% NCT in 5% peptone after 60 min. Organic matter had no effect on the activity of GSE. Moreover, the minimal concentration of BAT and GSE to inactivate viruses was lower than that known to kill human cells. It was concluded that the three compounds have potential to be used for water disinfection for drinking or reuse purposes.

## **11 Estimating the Infectivity of Human Norovirus and Other Single-Stranded RNA Viruses through Low-Pressure UV Disinfection**

Nicole Rockey, Suzanne Young, Brian Pecson, Christiane Wobus, Lutgarde Raskin, Tamar Kohn, Krista R. Wigginton

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One of the main challenges in water reuse is the adequate removal of microbiological hazards. Viral pathogen removal is of primary concern because viruses can be present in elevated concentrations in wastewater and are highly infectious. Yet the fate of certain viruses through reuse processes is not well characterized. Human norovirus (HuNoV), for example, is of principal interest in reuse because of its large burden of disease and ubiquitous presence in wastewater. Unfortunately, HuNoV infectivity through treatment remains elusive due to the absence of robust methods that measure infective HuNoV reliably (e.g., culturing). In this study, we have identified two approaches to track UV inactivation of HuNoV and other positive-sense (+) single-stranded RNA (ssRNA) viruses without relying on culture methods. First, we applied a framework for tracking UV degradation that relies on genome extrapolation of qPCR results. Our results suggest approximately 4-log inactivation of HuNoV at a UV dose of 27 mJ/cm<sup>2</sup>. These results display that HuNoV is inactivated similarly to other (+) ssRNA viruses, including murine norovirus, feline calicivirus, and echovirus 11, but is more susceptible to UV degradation than MS2, a commonly used surrogate.

We have also assessed whether the inactivation of (+) ssRNA viruses could be determined using genome sequence information, and thus negate the need for costly culture-based methods or qPCR-based methods. Specifically, we developed an inactivation model based solely on variables concerning nucleic acid composition (e.g., number of pyrimidine bases, neighboring pyrimidine bases, etc.). The resulting method correctly predicts inactivation rates of various (+) ssRNA viruses. These results on the inactivation of HuNoV and other RNA viruses can be applied to reuse treatment schemes to ensure the risks associated with reuse are being appropriately assessed.

## 12 Natural Viruses for Monitoring the integrity of Reverse Osmosis Membranes

Luc M. Hornstra, Tania Rodrigues da Silva, Bastiaan Blankert, Leo Heijnen, Erwin Beerendonk, Emile Cornelissen and Gertjan Medema

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High pressure membranes are increasingly used for the treatment of contaminated water to water with various purposes including irrigation and drinking water. The lack of a fast and easy to implement membrane integrity test method with a 10Log Removal Value (LRV) > 3 hampers the implementation of these membranes. Current online methods include conductivity, TOC and turbidity and can monitor a maximum LRV of 3. Challenge tests using virus surrogates such as bacteriophage MS2 show that membrane systems can reach LRV of 7, but dosing of these surrogates is not desirable in full scale drinking water plants. This study describes the identification and use of indigenous naturally present viruses, to monitor the integrity of RO membranes. Natural viruses were identified from fresh source water using metagenomics, and Q-PCR assays to detect these viruses were used to determine the gene copy number of these viruses in the feed and permeate of a pilot RO installation. The LRV of these natural viruses was compared with the LRV of spiked MS2 and online conductivity. The selected natural viruses demonstrated a LRV of > 7 and the results were similar to the results of spiked MS2 bacteriophage. Furthermore, after inflicting damage to the membrane element by drill holes, both MS2 and the natural viruses detected the damage to the membrane with a nearly identical loss of LRV, while conductivity lacked sensitivity to monitor any integrity loss. This novel method enables monitoring RO membrane integrity at a high sensitivity (LRV > 7), without the addition of chemical or biological virus surrogates. Furthermore, the high concentration of viruses in source water simplifies detection without laborious sample concentration procedures, and facilitates monitoring of the integrity of RO membranes in full scale operation with a much higher sensitivity than current methods.

## 13 Bacterial Identification by MALDI-TOF MS for routine management of a Drinking Water Treatment Plant

Anna Pinar-Méndez (1, 2), Belén Galofré (1), Anicet R. Blanch (2), Cristina García-Aljaro (2)

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Preventing strategies to avoid microbial contamination in water treatment systems require rapid and reliable detection to reduce or eliminate potential risks to the consumer's health. While bacteriological monitoring and testing, according to the Directive 98/83/EC, grant the detection of pathogenic bacteria in drinking water systems, newer and fast methods are needed to detect variations of bacterial communities that may influence water quality. *Matrix-Assisted Laser Desorption and Ionization Time-of-Flight Mass Spectrometry* (MALDI-TOF MS) is a fast technique currently used for clinically relevant bacterial identification based on proteomic fingerprinting, however, specific databases or in-house library building for environmental samples are required.

In the present study, a MALDI-TOF MS identification routine analysis has been implemented to

monitor cultivable heterotrophic bacteria throughout a full-scale drinking water treatment plant in Sant Joan Despí (Barcelona, Spain). At present, 1,265 colonies have been isolated from 45 samples collected at nine different treatment steps: raw river water and groundwater, decantation, sand filtration, ozonization, carbon filtration, reverse osmosis, mixed chamber and post-chlorination water. The identification and classification have been performed simultaneously using two databases: Bruker library (Bruker Daltonics, Bremen, Germany) and a specific library of drinking water bacteria developed during a previous project (Drinking Water Library Project, RTC-2015-4496-2).

The main results showed a 60-70% isolates successfully identified according to both databases, with 49 different genera represented.

Changes in bacterial diversity were observed throughout the whole treatment steps, from high diverse bacterial communities in river and groundwater to a significant decrease in diversity after chlorination step, where *Bacillus* sp. was the most prevalent genera.

This method provides a useful tool to characterize bacterial populations, monitor their fluctuations in drinking water systems, and detect possible failures at the different treatment steps, which is expected to be valuable for water production management.

#### **14 Characterizing changes to opportunistic pathogen concentrations in a full-scale, multi-step ozone-biological filtration drinking water treatment plant**

Katherine Dowdell, Oliver Köster, Urs von Gunten, Lindsay Caverly, John LiPuma, Frederik Hammes and Lutgarde Raskin

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Nontuberculous mycobacteria (NTM) are increasingly linked to respiratory infections, particularly in susceptible populations. It is believed that drinking water is an important source of acquisition, and clinically-relevant NTM have been reported at significant concentrations in drinking water and associated biofilms. Certain NTM are resistant to common drinking water disinfection processes. The lack of NTM monitoring and reporting in water, and the lack of resolution to the species or strain level in past research, make the assessment of health risks associated with NTM in water difficult to assess. Recently, Kotlarz et al. reported that NTM were capable of surviving ozonation and growing within a biologically active, granular activated carbon (BAC) filter at a drinking water treatment plant (*Environ Sci Technol* 52(5):2618-2628, 2018). We hypothesize that ozonation prior to biofiltration may select for oxidant-resistant species, such as certain NTM. To evaluate the impacts of treatment on the water microbial community, water and filter media samples were collected from a full-scale ozone-biofiltration plant, where treatment consists of ozone-rapid sand filtration-ozone-BAC-slow sand filtration. Molecular methods, including qPCR targeting the NTM *atpE* gene and the bacterial 16S rRNA gene, reverse transcription (RT)-qPCR targeting the NTM *mce1* gene, and sequencing of bacterial 16S rRNA genes and the NTM *rpoB* gene are being used in addition to flow cytometry and traditional culture-based methods to characterize changes to bacterial populations during treatment. Results show considerable changes to intact and total cell counts and community composition with each treatment step. Flow cytometry shows that, although there is substantial loss of viable cells after ozonation, some cells survive, particularly after the first ozonation stage. Molecular work, to be completed mid-2019, will allow for the identification of surviving groups and provide insight into how ozonation and biofiltration impact NTM and the larger community.

## **15 Is more, better? Disinfection residuals impact biofilms and water quality in drinking water distribution systems**

Katherine E. Fish, Joby Boxall

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Residual chlorine concentrations are often maintained in drinking water to mitigate planktonic microbial contamination and associated water quality and public health risks during its delivery through the drinking water distribution system (DWDS). However, the DWDS microbial load is primarily within biofilms - microbial communities adhered to the pipe walls via extracellular polymeric substances. Biofilms impact water quality by their activity and/or their mobilisation into the water column. Idealised, bench-top studies indicate that biofilms have increased tolerance to disinfection compared to their planktonic counterparts. However, the impact of chlorine residuals upon DWDS biofilms and the subsequent risk they present to water quality is unclear. To address this an internationally unique full-scale DWDS test facility, fed with water from the local DWDS, was used to grow biofilms (for 28 days) under three chlorine regimes Dechlorinated (low/no-), Medium- and High-chlorine. Various water quality parameters were monitored throughout the experiment and biofilms were sampled at days 0, 14 and 28 (n=5). After growth a “mobilisation” test was conducted that simulated the hydraulic changes that occur in DWDS following increases in water demand or a burst, which cause a release of material from the pipe walls leading to water quality failures. During “mobilisation”, biofilms conditioned to each chlorine regime were exposed to incremental increases in shear stress to determine any water quality degradation as a consequence of microbial release from the biofilms. High-chlorine reduced biofilm bacterial concentrations, selected for unique bacterial and fungal communities, and resulted in the greatest decrease in water quality, in response to mobilisation. The unanticipated findings suggest chlorine-boosting should be considered carefully and may actually exacerbate water quality issues, which have the potential to endanger public health. The derived understanding could impact the long-term management of DWDS water quality and biofilm, whilst challenging the mind-set of disinfection control strategies.

## **SESSION 3 – SURVIVAL, PERSISTENCE, TRANSPORT, FATE AND OCCURENCE**

### **16 Precipitation and Salmonellosis Incidence in Georgia, United States of America: Interactions Between Extreme Rainfall Events and Antecedent Rainfall Conditions**

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Compared to other regions of the United States, the southeast consistently has high salmonellosis incidence rates, but the drivers of disease remain unknown. *Salmonella* is regularly detected in the natural environment in this region, leading to numerous exposure opportunities. Rainfall patterns may impact the survival and transport of *Salmonella* in the environment in ways that can affect disease transmission. This study investigated the impacts of short-term precipitation (extreme rainfall events above the 90th percentile of daily rainfall) and longer-term precipitation (rainfall conditions antecedent to these extreme events, categorized into tertiles of eight-week sums of daily precipitation) on county-level salmonellosis counts using negative binomial models. We found that in the counties of the Coastal Plain of Georgia, where *Salmonella* is frequently detected in the environment, extreme and antecedent rainfall conditions had significant impacts on salmonellosis counts. Specifically, in these counties, extreme rainfall was associated with a 5% increase in salmonellosis risk (95% CI: 1-10%) compared to weeks with no extreme rainfall. Antecedent dry periods were associated with a 9% decrease in risk (95% CI: 5-12%) while antecedent wet periods were associated with a 5% increase in risk (95% CI: 1-9%), compared to antecedent periods of moderate rainfall. In models considering the

interaction between extreme and antecedent rainfall, extreme rainfall events were associated with an 11% increase in risk (95% CI: 5-18%) when they occurred following moderate rainfall or wet periods. These estimated effects were substantially magnified when restricting analyses to salmonellosis cases attributed to serovars commonly isolated from wildlife and the environment (e.g. Javiana). Given the observed impact of short-term extreme rainfall events and longer-term rainfall conditions on salmonellosis incidence, efforts to inform the public of the risks of contact with the environment following heavy rainfall events, especially during the rainy season, may be an important aspect of prevention.

## 17 Persistence of Microbial Source Tracking markers, *E. coli* genotypes and fecal indicator bacteria in seawater and freshwater microcosms

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Natural seawater and freshwater microcosms were inoculated with either wastewaters, cowpats, poultry feces, or a combination of the three to determine the persistence of human, bovine and bird Microbial Source Tracking markers in these two types of surface water.

Both concentrations of host-associated bacterial qPCR markers (i.e. HF183, Rum2Bac, GFD, AV4143, and AV43 markers) and evolution of *E. coli* genotypes identified by ERIC-PCR were analyzed for 18 days along with the concentrations of fecal indicator bacteria (FIB). The feces and wastewater were inoculated to unfiltered water microcosm incubated aerobically at 18°C in the dark.

The human-associated HF183 marker persists for seven days in both marine and freshwater, showing a weaker persistence than FIB. The ruminant-associated Rum2Bac marker persists for the entire experiment (18 days) in seawater, i.e. similar to FIB and seven days in freshwater microcosms, i.e. less than FIB. The general bird markers GFD and AV4143 persist for the 18 days of the experiment, same duration as FIB, whereas the poultry-associated AV43 marker persists for nine days in both water types.

Focusing on the *E. coli* FIB, T90 are lower in seawater than in freshwater, with the exception of the poultry feces experiment. Genotypes composition also differs in fresh and marine waters (<33% of common genotypes). However, *E. coli* populations exhibit the same general pattern of persistence regardless of both water type and feces origin. Most persisting isolates (>55%) belong to a limited group of genotypes (≤ 33% of the genotype richness in all but one experiment) evenly detected for 18 days. In our experimental setup FIB persistence is likely to be supported by a poorly diversified set of *E. coli* strains.

Although those results depend on the experimental conditions, this study provides essential data to validate the MST toolbox for the identification of fecal contamination in the environment.

## 18 Stability of PMMoV and Enteric Viruses in Tap Water Using Viability qPCR

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Pepper mild mottle virus (PMMoV) has been proposed as a useful viral indicator for source water as well as for fate of viruses during treatment processes. However, the indicator suitability of PMMoV to represent the existence of enteric viruses in tap water has not been well investigated. The aim of this study was to evaluate the occurrence and persistence of PMMoV and enteric viruses in tap water using

qPCR combined with cis-dichlorodiammineplatinum (CDDP) pretreatment (CDDP-qPCR), which can selectively quantify intact viruses. A total of 27 tap water samples (300–500 L) were collected in Kanto region, Japan and tested for the presence of pepper mild mottle virus (PMMoV) and human enteric viruses including Aichi virus (AiV), enteroviruses, noroviruses (GI and GII), adenoviruses, BK and JC polyomaviruses using normal qPCR and CDDP-qPCR. Among the viruses tested, PMMoV was the most frequently detected in 37% of samples with an average concentration of 285 copies/L, followed by AiV (7%, 9.6 copies/L) using normal qPCR, indicating that PMMoV was more abundant than other target viruses. In addition, when tested by CDDP-qPCR, PMMoV was still detected in 22% of samples with an average concentration of 16 copies/L while other target viruses were under the limit of detection (1.9–4.8 copies/L). The higher detection of PMMoV indicates that their capsid was more persistent than other target viruses. Thus, the absence of PMMoV can be a safety side proof to ensure the absence of human enteric viruses in tap water.

## **19 Systematic Review and Meta-Analysis of Waterborne Mammalian Viruses and Coliphage Decay Rate Constants in Surface Waters**

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Viruses are among the most infectious water-related pathogens and a leading cause of gastrointestinal illness among the world's population. We conducted a systematic review of the literature, following best practices, to gather decay rate constants ( $k$ ) of mammalian waterborne viruses (enteroviruses, adenoviruses, noroviruses, astroviruses, rotavirus, and hepatitis A viruses) and coliphage in raw surface waters to aid in the parameterization of virus fate and transport models. We identified 562  $k$  values in the literature with the largest number identified for enteroviruses and coliphage and the smallest for astrovirus, hepatitis A, and norovirus. Values varied from 0.07 to 0.9 per day, on average, in order from smallest to largest: norovirus < astrovirus < adenovirus < hepatitis A < rotavirus < coliphage < enterovirus. A meta-analysis investigated how  $k$  values varied among viruses for experiments conducted with different virus serotypes or species at different temperatures, salinities, and sunlight exposures, and for experiments that enumerated viruses using different methodologies. Virus species or serotype did not affect  $k$  among decay experiments, hence viruses are hereafter referred to using their common taxonomic assignment.  $k$  values were generally larger for experiments conducted at higher temperatures, in sunlight, and in estuarine waters, and enumerated using culture methods.  $k$  values were statistically different between virus types with *Norwalk virus*, *Hepatovirus A*, and *Mastadenovirus* having smaller  $k$  values than other viruses, controlling for experimental condition and enumeration method. While F+ coliphage  $k$  values were similar to those of *Enterovirus*, *Human astrovirus*, and *Rotavirus A*, they were different from those of the other mammalian viruses. This compilation virus  $k$  values provides essential information for researchers and risk assessors modeling virus fate and transport in surface waters and identifies avenues for future research.

## **20 Dynamics of crAssphage as a human source tracking marker in potentially faecally polluted**

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Recently, an abundant bacteriophage called crAssphage has been described in human faecal samples from many places. Different studies propose crAssphage as a potential human-associated Microbial Source Tracking (MST) marker in water, although its effective implementation in water management strategies will depend on how it behaves in the environment. The objective of this work was to study the dynamics of crAssphage in the environment evaluating its potential use as human-associated MST marker. We analyzed the abundance and temporal distribution of crAssphage in sewage effluent of 19 plants with different treatments, and 2 rivers: i) Llobregat River (high levels of fecal pollution and

intermediate annual flow) and ii) Riera de Cànoves, (low levels of fecal pollution and a low annual flow). Moreover, the influence of climatic conditions such as temperature and precipitation has been studied on the inactivation of the marker. Natural inactivation has been monitored: i) along a section of the Riera de Cànoves receiving the sewage effluent discharged into the river; ii) following a mesocosms approach. The concentration and dynamics of crAssphage have been compared with indicators of bacterial and viral general fecal contamination: *E. coli* and somatic coliphages, and human-associated MST markers such as bacteriophages of *Bacteroides* strain GA17, and the molecular markers HF183 and HMBif. The concentrations of crAssphage in wastewater effluents and river samples were similar to those of molecular markers and higher than general indicators (*E. coli*, somatic coliphages) and GA17PH. No temporal differences were observed in the effluent or in the rivers throughout the year and fluctuations in their abundance are related to rainfall events, although higher temperature increases its inactivation. The observed dynamics of crAssphage in the environment supports its use as human-associated MST marker, since despite having a similar abundance to other molecular human MST markers, it showed longer environmental persistence.

## SESSION 3 – RISK FOR WASTE WATER TREATMENT PLANT WORKERS

### 21 Protecting Wastewater Workers from Microbial Risks: Personal Protective Equipment Guidelines

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The Occupational Safety and Health Administration requires that employers evaluate workplace hazards and institute measures to prevent employee exposure to recognized risks. A job safety analysis (JSA) is a systematic procedure to integrate accepted safety and health principles and practices into particular tasks or job operations. Based on the JSA, appropriate PPE can be selected to protect the worker from recognized hazards. However, guidelines on use of JSAs and appropriate PPE in the wastewater industry are lacking.

A workshop of 34 wastewater utility personnel and public health experts participated in structured small group discussion sessions to develop recommendations for PPE for wastewater workers for 43 typical job activities grouped into six work environments and developed preliminary recommendations for minimum levels of PPE to protect workers from exposure to biological risks.

At a minimum, wastewater and collection system workers should don PPE to prevent exposure to infectious agents via contact transfer, such as durable gloves, for all job activities assessed. Protection against splashing, such as safety glasses, goggles, or face shields is recommended for 24 of 43 job activities. Tyvek suites or coveralls, is recommended for 4 of 43 job activities. Respiratory protection, such as an N95 mask, is recommended for 10 of 43 job activities to prevent inhalation of infectious agents.

Five primary research needs were identified:

1. Conduct a prospective epidemiological study of infectious disease incidence among wastewater workers.
2. Characterize respiratory exposure for typical tasks performed in wastewater operations.
3. Characterize contact exposure for typical tasks.
4. Perform cost-benefit analyses of PPE for wastewater workers.
5. Refine guidance and develop best practices

The results will support the development of an evidence-based protocol to minimize infectious disease risk for wastewater and collection system workers. The PPE selection matrix can serve as a guide for protecting over 136,000 wastewater workers.

## **22 Biological risks for workers of WWTPs: overview, methods and tools for risk assessment**

Annalaura Carducci, Ileana Federigi and Marco Verani

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Wastewater treatment plants of domestic sewages represent a collection point of pathogenic microorganisms circulating in a definite population. Workers in these plants can be largely exposed to these microorganisms by inhaling bioaerosol or having contacts with contaminated surfaces/objects and develop symptomatic or asymptomatic infections, thus representing possible sources of infection for general population.

The biological risk assessment for workers in WWTP is generally based on a qualitative approach, using (at the maximum) fecal indicators.

Besides the evidence of workers infections deriving from epidemiological studies, several field monitoring indicated the most relevant sources of bioaerosol in WWTP and the spreading of microbial contamination through air. They demonstrated that the bioaerosol production is related to the step and the technology of the plant, but also that technology improvements and measures of confinement resulted in a clear abatement of contamination. Studies including pathogens in monitoring also demonstrated that the use of bacterial fecal indicators to evaluate the exposure was clearly insufficient in particular for virus. On the other hand, the choice of index pathogens as well as the one of methods for sampling and sample processing, is quite difficult.

The presentation about occupational biological risks in WWTP will focus on the approach for risk assessment and the related criticisms in hazards identification, exposure evaluation/measurement, risk characterization and assessment. To this aim, data from field experience in WWTP will be analyzed and included in a wider perspective of international studies. Finally a QMRA model will be proposed and discussed. This approach is not yet common for working settings and it could be very useful in order to prioritize and define the control measures needed to reduce the risk.

## **SESSION 4 – MODELLING AND PREDICTION**

### **23 Can stochastic models predict microbial peak events?**

Émile Sylvestre, Jean-Baptiste Burnet, Patrick Smeets, Gertjan Medema, Michèle Prévost, Sarah Dorner

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Water providers are encouraged to implement source water monitoring programs that capture the most important sources of variation in microbiological source water quality. The collection of event-based samples allows evaluating if stochastic models developed with routine monitoring data correctly predict microbial peak concentrations. The goal of our study was to develop a generic approach to integrate this verification in an exposure assessment.

We collected high-frequency event-based (during rainfall and snowmelt events) and routine monitoring data of *Cryptosporidium* and *Giardia* at three drinking water treatment plants (two urban and one agricultural) in Quebec, Canada. Poisson, negative binomial, Poisson log-normal, and Poisson inverse-gaussian distributions were fitted on monthly samples collected over two years ( $n=24$ ). The goodness-of-fit of each model was measured with the deviance information criterion (DIC). Complementary cumulative distribution function (CCDF) curves were used to illustrate the behavior of the tail of these distributions. The maximum average daily concentrations obtained with event-based sampling were compared to the tails of the best-fit models.

The Poisson log-normal distribution best described the variability of *Cryptosporidium* and *Giardia* concentrations. The range of the uncertainty on the mean *Cryptosporidium* concentration of the best-fit model varied between 0.6 log10 and 3.2 log10. Maximum average daily concentrations obtained with event-based sampling were bounded by the tail of the best-fit distribution at each DWTP. Therefore, these peaks were predicted by the stochastic model, and were already included in an exposure assessment with routine monitoring data. Event-based samples could be used to develop distributions with truncated tail to reduce large uncertainties on the mean concentration at one of these DWTPs.

## 24 Fully-integrated model for *E. coli* dynamics in urban estuaries

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Modelling of *E. coli* dynamics in urban estuaries at a fine temporal scale is complex and requires an integrated modelling approach. In this study, modelling of *E. coli* inputs was achieved using an existing model, MOPUS, while a new three-dimensional hydrodynamic-microorganism model was developed for modelling estuarine processes. The estuarine microorganism model simulates three fractions of *E. coli*: free *E. coli*, sediment-attached *E. coli* and *E. coli* deposited in the bed sediments. Die-off is modelled dynamically as a function of water temperature, salinity and sunlight. The model also accounts for settling and resuspension of sediment-attached *E. coli*. The model was tested on the Yarra River estuary using extensive dataset of more than 3500 measured *E. coli* concentrations from two locations in the estuary as well as more than 80 *E. coli* depth profiles. Sensitivity analysis revealed that the exclusion of die-off and sediment-microorganism interaction had minimal effect on the predictive capability of the model (ELOG = 0.22 vs. ELOG = 0.29), suggesting that *E. coli* dynamics in the Yarra River estuary is driven by inputs and hydrodynamic transport and mixing. The importance of the Yarra River freshwater input was further confirmed by using measured datasets (instead of modelled ones) which led to a significant increase in the model's predictive performance (ELOG = 0.18 vs. ELOG = 0.38). To explore model structural uncertainties, the performance of a simple conceptual spatially-lumped microorganism model was tested (ELOG = 0.41), suggesting that a simpler model could represent the dataset to a similar efficiency level as the new three-dimensional hydrodynamic-microorganism model. While simpler models can be used to provide quick insights into microbial dynamics (e.g. use in an early warning system at a particular location), the process-based models, due to the complexity they incorporate and the amount of output information they produce, are still more valuable for informing mitigation strategies.

## 25 Recognizing what cannot be known from available data: Structural non-identifiability and its implications in QMRA and beyond

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Goodness of fit is the chief concern in purely empirical statistical modelling, but must not be the only concern in theory-based statistical models. When the parameters and distributions of a model are believed to represent practically meaningful phenomena, structural identifiability is also critically important. Identifiability concerns the quality of objective information in the available data to facilitate estimation of a parameter; a structurally non-identifiable model has parameters for which the data provide little or no information. Many problems can arise when structural non-identifiability is overlooked by modellers, possibly undermining statistical inferences obtained using developed models.

This study explores structural non-identifiability and its implications in the context of quantitative microbial risk assessment. Some experimental designs can produce data that are inherently incapable of yielding objective information about important model parameters, and it is better to know this in the

design stage so that wasteful experimentation can be averted. This is shown using an example based upon temporally distributed presence/absence data. Conversely, available data may be inherently insufficient for fitting a model of a particular form. An example is provided in which particular *E. coli* O157:H7 outbreak data cannot be used to fit an exact beta-Poisson dose-response model but for the overly informative subjective information in Bayesian analysis. Finally, when model parameters are non-identifiable, there can be a suite of spurious models featuring excellent fit to the data but misleading statistical inferences. An example based upon a norovirus dose-response experiment featuring uncontrolled aggregation in the viruses administered to humans is considered. Knowledge of structural non-identifiability is essential to discerning when certain experiments should not be conducted, when it may be inappropriate to use Bayesian methods in model-fitting, and when a poorly justified assumption could lead to a spurious model.

## **26      Implementation of reliable early warning systems at European bathing waters using multivariate Bayesian regression modelling**

Wolfgang Seis (\*, \*\*), Malte Zamzow (\*), Pascale Rouault (\*)

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For ensuring microbial safety at bathing waters, which are subject to short-term pollution, the current European bathing water directive (BWD) (76/160/EEC 2006) demands the implementation of reliable early warning systems to timely inform the population about contamination episodes prior to human exposure. Besides novel approaches for rapid microbial analysis, predictive modelling approaches are promising solutions for achieving this objective.

Challenges regarding the implementation of reliable model-based early warning systems are the lack of explicitly defined decision-criteria in the BWD, as well as in the lack of reliable data sets for both model calibration and validation. In the present study, we developed statistical prediction models for predicting *E.coli* concentrations at a river bathing location affected by periodic contamination episodes caused CSO discharges in Berlin, Germany. Models were developed based on readily available hydraulic data (rainfall, flow, WWTP discharges). We furthermore developed new decision, model validation and selection criteria, which account for the probabilistic character of the BWD's approach of long-term classification and for potentially unreliable information in official surveillance data due to present sampling uncertainty. Model predictions were validated from 2016-2018 with currently over 600 samples including hourly grab samples ( $N = 400$ , 2018 unpublished), event-based 12-composite-samples ( $N = 153$ , published in Seis et al. (2018)) and data from official bathing water surveillance ( $N = 50$ , partly published in Seis et al. (2018)). Validation monitoring will continue in 2019 (planned  $N = 400$ ) with data being available until September 2019. Validation monitoring showed that prediction modelling is able to reliable predict contamination episodes and demonstrated to improve health protection.

Seis, W., Zamzow, M., Caradot, N. and Rouault, P. (2018) On the implementation of reliable early warning systems at European bathing waters using multivariate Bayesian regression modelling. Water Research 143, 301-312

## **27      Modelling the transport, immobilization and remobilization of contaminant microorganisms in streams accounting for hyporheic exchange and dynamic flow conditions**

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Rivers transport contaminant microorganisms (including faecal indicator bacteria and human enteric bacteria and viruses) long distances downstream of non-point and point sources, posing a direct risk for human health. During baseflow conditions, hyporheic exchange flow processes between surface

water and the streambed transport microorganisms into streambed sediments. Microorganisms immobilize and accumulate within streambed sediments during baseflow conditions by gravitational settling, attachment to in-stream structures such as submerged macrophytes, and hyporheic exchange and filtration within underlying sediments. However, dynamic flow conditions alter the transport processes of contaminant microorganisms, notably by remobilizing them during (bed-sediment disturbing) high flow events. The extent of remobilization is dependent on both, the increase in magnitude of the flow and the number of microbes previously immobilized within the streambed sediments. Therefore, in order to predict the persistence and fate of contaminant microorganisms in streams it is important to consider the dynamic variations between baseflow and high flow conditions in a model framework. We present a mobile-immobile model that incorporates contaminant microbe transport, its immobilization and inactivation during baseflow, and remobilization during high flows. The model considers antecedent conditions by incorporating the time since the last remobilization event and the magnitude of the increased flow. Model results are validated by mesocosm and field (stream and streambed) observations of *Escherichia coli* and fine particles under varying flow conditions. Model outputs were able to closely match observations of *E. coli* and fine particles through multiple high flow events. Incorporating flow variations in the model enables for more realistic characterization of contaminant microbe dynamics within stream environments and supports predictions of their downstream transport and impacts.

## **28           The impact of the wastewater treatment plants on the virus concentrations in surface waters in the Netherlands**

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The objective of this study was to develop a model of emission, transport and fate of enteric viruses from treated (and untreated) wastewater discharges that predicts the concentration of viruses in all major surface waters in the Netherlands. WFD Explorer 2.0 is a national water balance model that schematizes the waterways in 2575 surface water units (SWUs) and includes emissions from 345 wastewater treatment plants and transboundary import of water. Virus decay was incorporated as an exponential model. Quarterly averaged water balance data from an extreme dry and wet season were used to incorporate climate variability, i.e. the 3rd quarter of 2003 and the 4th quarter of 1998 respectively. Data on norovirus GGI and II (qPCR), adenovirus (qPCR) and enterovirus (cell culture) concentrations in domestic wastewater, their removal by conventional wastewater treatment, and survival in water at different temperatures were compiled and used to populate the model. The output was maps of modelled virus concentrations in all SWUs in the Netherlands. For surface water sites where culturable enterovirus concentration were available, the modelled concentrations at these locations were in the same order of magnitude. The virus concentration at a specific location was determined by a combination of input, virus characteristics and hydrology. Virus concentrations were higher in the dry period for most of SWUs. Virus concentrations were lower at sites with long residence times, most pronounced for enterovirus that decay more rapidly. The model allowed the determination of the most significant sources of virus contamination at specific sites, such as sites used for drinking water production, bathing or shellfish farming. It also allows to conduct scenario studies to determine the impact of additional control measures or studies to evaluate the impact of global change scenarios, such as climate change.

**29           Modelling *Legionella* transmission from wastewater treatment plants in the Netherlands**

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**Introduction:** Endemic Legionnaires disease (LD) incidence has risen in the Netherlands, and in the majority of cases the source of infection is never found. Wastewater treatment plants have been identified as a potential source [1]. This study aims to apply spatial modelling to identify environmental sources of *Legionella* infections, with a specific focus on wastewater treatment plants as a source.

**Methods:** A combination of two modelling approaches is presented. First, a source detection model developed for Q fever was adjusted for *Legionella* [2, 3]. This model predicts source location given LD cases. Secondly, an aerial transmission model (OPS) [4] developed for particle transport is applied for aerosolized *Legionella*. This model predicts the location of LD cases given a source. Further methodological development of these approaches is presented.

**Results:** The source detection model has successfully been applied for an LD cluster linked to a wastewater treatment plant [2], as well as a cooling tower-related outbreak. The model was able to identify the source location within a few 100 meters of accuracy, based on a limited number of cases and simple input data.

**Conclusion:** Biological wastewater treatment plants, both of domestic and industrial wastewater, are a proven source of LD cases. Source detection modelling and aerial dispersion modelling are promising techniques to understand environmental transmission of *Legionella*.

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2. Loenenbach, A.D., et al., *Two Community Clusters of Legionnaires' Disease Directly Linked to a Biologic Wastewater Treatment Plant, the Netherlands*. Emerg Infect Dis, 2018. **24**(10): p. 1914-1918.
3. van Leuken, J.P., et al., *A model for the early identification of sources of airborne pathogens in an outdoor environment*. PLoS One, 2013. **8**(12): p. e80412.
4. Sauter, F., et al. *OPS model - Description of OPS 4.5.2*. 2018 [cited 2019 19 March]; Available from: <https://www.rivm.nl/media/ops/OPS-model.pdf>.

**SESSION 5 – MICROBIAL FECAL POLLUTION DIAGNOSTICS AND SOURCE TRACKING**

**30           Microbial Source Tracking at Chicago Beaches under Dry and Wet Weather Conditions**

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Fecal bacteria concentrations are often reported to increase in surface waters after heavy rainfall mostly due to local municipal stormwater and sewer conveyance discharges. Chicago area Lake Michigan beaches present a unique scenario where stormwater and wastewater flows are engineered to discharge in the nearby Chicago River diverting pollution away from the lake and its recreational sites. Therefore, under dry and most wet weather conditions, these beaches should not be impacted by point

sources of fecal contamination. Yet several Chicago beaches often exceed the U.S. Environmental Protection Agency recommended Beach Action Values (BAV). Here we investigate the potential influence of rainfall on general fecal indicator bacteria (FIB) and microbial source tracking (MST) genetic markers at non-point source impacted Chicago beaches. During the summer of 2016, water samples were collected at nine Chicago beaches and analyzed for *E. coli* by culture and enterococci by quantitative polymerase chain reaction (qPCR). Select samples ( $n = 195$ ) were tested for human (HF183/BacR287, HumM2), canine (DG3, DG37), and avian (GFD) fecal source sources. We then examined the occurrence and concentrations of general FIB and MST markers under wet and dry weather conditions. Precipitation tended to increase concentrations of both enterococci and *E. coli*. Following rainfall, the odds of BAV exceedance increased based on *E. coli* results, but not enterococci. Using a weighted-average fecal score approach, we observed that the MST marker concentration (log<sub>10</sub> copies per 100 mL) for DG3 was 2.4 times higher, DG37 was 2.1 times higher and GFD was 1.6 times higher during wet compared to dry weather conditions. In contrast, HF183/BacR287 average concentrations were not significantly different ( $p > 0.05$ ), regardless of weather conditions. MST findings coupled with precipitation information offer valuable new insights regarding the role of non-point stormwater and recreational beach water quality management for better health protection of beachgoers.

### **31 Examining Relationships Between Physio-chemical and Microbiological Source Tracking (MST) Water Quality Parameters Along with Agricultural Land Use at the Watershed Scale**

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The goal of this study was to evaluate the relationships between non-point sources of fecal contamination, water chemistry, land use, and MST markers. Water samples ( $n=136$ ) were collected from 17 sites from 5 watersheds in Michigan's Lower Peninsula during eight sampling events, between April 2017 and May of 2018, representing the growing season, fall/winter baseflow and spring snow melt. We tested whether streamflow and water quality parameters were related to concentrations of *E. coli* and three MST markers (human, bovine, and porcine). We used Spearman's rank-order correlation method to remove highly correlated water quality parameters and reduce collinearity. Multivariate analysis was used to identify spatial and temporal clusters for the microbiological data. The resulting spatial clusters were consistent with land use and tillage practices at each watershed, whereas temporal clusters were mainly explained by hydrological conditions. We identified two major spatial clusters of sampling locations, which resembled the difference in the percentage of the developed area within each watershed. The proportion of explained variance for each microbiological parameter improved when considering the clusters instead of using all the data at once. We then used the Soil and Water Assessment Tool (SWAT) to estimate streamflow and nutrients at each sampling location to develop predictive models to quantify the effects of agricultural management practices. The relative importance of the explanatory variables in the model varied with each cluster. Moreover, no single variable was found to be dominant among all clusters. This shows that while nutrient and microbiological impacts can be modeled in each watershed, disparity in explanatory variables prevents a generalized model from being created for all watersheds.

## **32 Building Microbial Source Tracking Capacity to Address Nonpoint Sources of Fecal Pollution at Michigan Public Recreational Beaches**

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Public beaches are posted with closures or advisories when monitoring results of *Escherichia coli* (*E. coli*) exceed water quality standards in the State of Michigan. However, this approach does not address the sources of *E. coli* that cause beach closures. A review of 24 years of beach closure data on the BeachGuard website managed by the State of Michigan demonstrated that 70% of 1495 closures were reported with “unknown” causes of high *E. coli* levels. This problem underscored the need for microbial source tracking (MST) to identify nonpoint sources of fecal pollution at these public beaches. In response to this problem, three beaches (Luna Pier, Billings Lake, and Huron – Clinton Metropark) were monitored for fecal pollution using human, cow and gull qPCR assays. Human fecal pollution was detected at all three beaches, while gull marker was detected at Luna Pier beach, and cow marker at Billings and Huron – Clinton Metropark beaches. These findings support the use of MST as a tool to help beach managers take corrective actions and eliminate sources. Ten laboratories were trained by Michigan State University and the United States Environmental Protection Agency, Office of Research and Development in 2018 to apply MST to identify human fecal sources and provide actionable information for impaired beaches. The State of Michigan is also initiating a network of MST experts in the region to apply MST at the watershed scale and share lessons learned and results across the Great Lakes region. This MST program of the State of Michigan is increasing awareness of the role of MST in addressing fecal pollution related challenges at local beaches and watersheds.

## **33 The Clean Water Experiment: microbial water quality monitoring by citizens in Amsterdam**

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Deltares, Waternet

Water quality in European cities is improving as a result of a decrease of industrial activities and water management measures. Citizens are increasingly using surface water for swimming, canoeing, or watering their garden. Information and knowledge for the general public is, however, limited. In Amsterdam, in the summer of 2017, a few hundred citizens monitored the famous canals, urban lakes, rivers and ditches in their city. Over 200 active participants assessed a variety of water quality parameters using a specially designed Waterbox. For assessment of fecal pollution, Waterbox contained petridishes with *E.coli*-specific agar and Aquagenx Compartment Bag Tests. In a 6-weeks period over 600 *E.coli*-analyses were uploaded and presented on the online citymap. All the data from the Clean Water Experiment are open for everyone to access. Besides the results, information about health risks and guidelines for microbial water quality were shared with the participants. Results of *E.coli*-specific agar incubations showed the average number of *E.coli* at 1.300 cfu/100ml, with 16% of the measurements above 1.800 cfu/100ml. Despite a high variety within the data, and examples of wrong and sub-optimal execution of the experiments, results reflected the direct relation between heavy rainfall and high fecal pollution in the canals.

Evaluation showed that 15% of the participants adapted their behavior after participation in the project. In most cases their recreational use of urban surface water increased while others decided not to swim anymore at a specific location. Citizen science can be a good way to make people aware on variations in microbial water quality, health risks, and effect of measures. With the lessons learned in 2017, we adapted the monitoring set-up and expect to improve the reliability of data. The Clean Water Experiment was continued in 2018 and started in other cities this year.

**34      The impact of major earthquakes and subsequent sewage discharges on the microbial water quality of an urban river in Christchurch, New Zealand**

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Following a series of earthquakes in Christchurch City in New Zealand (2011-2012), severe damage to the sewerage system resulted in discharge of large volumes of raw sewage into an urban river over a period of six months. This unusual occurrence, therefore, provided a rare opportunity to investigate the fate and transport of faecal contaminants discharged into a river system.

The study explored the relationships between faecal source tracking (FST) markers (quantitative Polymerase Chain Reaction (qPCR) markers and steroids and fluorescent whitening agents), microbial indicators, and potential human pathogens (*Giardia*, *Cryptosporidium* and *Campylobacter*). The results provided Health Authorities with information on the health risk associated with recreational activities in the urban river both during the sewage discharge period and post-discharge, which they disseminated to the general public through media releases and signage at river locations. It also alerted authorities to unexpected occurrences of faecal contamination caused by damage to sewer lines at locations upstream of the discharge sites. Subsequently, councils were able to track down leaking sewer pipes and perform remediation work.

Ongoing intermittent discharges due to earthquakes and aftershocks impacted on the study's ability to delineate timelines between the discharge and post-discharge phases, however, the study was able to highlight the persistence of pathogens and chemical markers in sediments. The results validated the use of *Escherichia coli* as a reliable indicator of public health risk. Importantly, the human source indicative FST markers demonstrated that they were effective predictors of pathogenic protozoa in water. This aspect affirmed the dual benefits of the FST toolbox in determining faecal source(s) and potential health risk. The statistically significant, substantial agreement between the two FST methods of qPCR and steroid analysis suggested that water managers could be confident in the results using either method.

**35      Poikilothermic animals as a previously unrecognized source of fecal indicator bacteria**

Christina Frick (a, b), Julia Vierheilig (c, d), Horst Zornig (e), Roswitha Antensteiner (f), Christian Baumgartner (g), Christian Bucher (h, i), Alfred P. Blaschke (d, i), Julia Derx (d, i), Alexander K.T. Kirschner (c, d, j), Gabriela Ryzinska-Paier (a), René Mayer (d, k), Dagmar Seidl (a), Theodossia Nadiotis-Tsaka (a), Regina Sommer (d, j), Andreas H. Farnleitner (c, d, k)

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Quantitative information regarding the presence of *Escherichia coli*, intestinal enterococci and *Clostridium perfringens* in poikilotherms is notably scarce. Therefore, this study was designed to allow a systematic comparison of the occurrence of these standard fecal indicator bacteria (SFIB) in the excreta of wild homeothermic (ruminants, boars, carnivores, birds) and poikilothermic animals (earthworms, gastropods, frogs, fish) inhabiting an alluvial backwater area in eastern Austria. With the exception of earthworms, the average concentrations of *E. coli* and enterococci in the excreta of poikilotherms were equal to or only slightly lower than those observed in homeothermic excreta and

were 1-4 orders of magnitude higher than the levels observed in the ambient soils and sediments. The median *E. coli* concentrations were 4.2 log<sub>10</sub> CFU/g in gastropods, and 4.7 log<sub>10</sub> CFU/g in poikilothermic vertebrates (fish, frogs). Enterococci reached extraordinarily high concentrations in gastropods (5.7 log<sub>10</sub> CFU/g), and a median concentration of 3.6 log<sub>10</sub> CFU/g in poikilothermic vertebrates. The median concentration of *C. perfringens* was 2.7 log<sub>10</sub> CFU/g in earthworms and gastropods, and 3.0 log<sub>10</sub> CFU/g in the poikilothermic vertebrates. Additional estimates of the daily excreted SFIB loads further supported the importance of poikilotherms as potential pollution sources. In conclusion, the long-standing hypothesis that only humans and homeothermic animals are primary sources of SFIB is challenged by the results of this study. It may be necessary to extend the fecal indicator concept by additionally considering poikilotherms as potentially important primary habitats of SFIB. These results do not imply that SFIB should not be used as indicators; however, they suggest that interpretation of these data is more complex than previously believed.

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### **36            Cross-country performance of a human-associated *E. coli* source tracking marker, H8, in Asia and Africa**

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Kyoto University, Hanoi University of Science and Technology, Makerere University, Khulna University, University of Zambia

Microbial source tracking genetic markers have gained global attention to identify fecal contamination sources in water environment. HF183 is a well-known human-associated marker targeting *Bacteroides* spp. and has been studied widely. Targeting *E. coli*, a new human-associated marker H8 was developed in Japan (Gomi et al., 2014). This study aimed at performance check of H8 in Bangladesh, Vietnam, Uganda and Zambia. In each country, we collected fecal samples from known-hosts including human, chicken, duck, goat, cattle, pig, and buffalo. After culturing *E. coli* on agar plates, maximum three isolates were picked from one sample and in total 275 and 1090 isolates were, respectively, collected from human and non-human samples. SYBR Green-based real-time PCR assay was performed using H8 for each isolate. Isolate-based performance of H8 was evaluated in terms of host specificity and sensitivity: specificity = true negatives / (true negatives + false positives), and sensitivity = true positives / (true positives + false negatives). Results show 77.1% specificity and 43.6% sensitivity for overall samples. This specificity is greatly lower than previous reports in Japan (99%, Gomi et al., 2014) and Australia (94%, Warish et al., 2015). Great variation of H8 performance was observed among the four countries. Specificity and sensitivity were, respectively, 91.7% and 16.4% for Bangladesh, 87.0% and 52.5% for Vietnam, 77.3% and 40.0% for Zambia, and 57.7% and 56.0% in Uganda. Only Vietnam showed significantly higher H8-positive proportion in human samples (52.5%) than in any types of other animal samples (1.3-20.1%). False positive proportions were significantly different even among same animal types: e.g., 32% in Uganda and 0% in Zambia for chicken. Although H8 performance was evaluated good in Japan and Australia, the performance seems substantially different in countries, suggesting careful application of H8 in a new region.

### **37      New approach for the detection of coliphages as indicators of viral fecal pollution in water**

Daniel Toribio-Avedillo, Julia Martín-Díaz, Anicet R. Blanch and Maite Muniesa

University of Barcelona

Two groups of coliphages have recently been included in different water management policies as indicators of viral fecal pollution: somatic coliphages, which infect *E. coli* through cell wall receptors, and F-specific RNA coliphages, which infect through the F-pili. In fecally polluted waters, somatic coliphages are the most abundant, with the exceptions of reclaimed water and some groundwater samples. Nevertheless, the use of strains sensitive to both types of phages is desirable for total coliphage enumeration, avoiding double analysis.

Standardized coliphage detection methods are robust and cost-effective, but require lengthy preparation and long incubation times. Thus, incorporating rapid and ready-to-use methodologies, like recently developed Bluephage®, would facilitate routine implementation in laboratories.

This approach uses several strains capable of detecting somatic coliphages (*E. coli* CB10 strain), F-specific coliphages (CB14 and CB16 strains, *E. coli* HS and *S. enterica* WG49 strains respectively expressing the F-pili) or both (CB12 *E. coli* strain). All strains were modified knocking out *uidB* and *uidC* genes, which encode the import of glucuronic acid, and overexpressing *uidA*, which encodes the β-glucuronidase. Since the strains cannot internalize the substrate, the glucuronidase accumulated in the cytoplasm only has contact with its substrate after cell lysis caused by phages. When the enzyme is released into the medium, it cleaves glucuronic acid linked to a chromogen, the chromogen is released and produces a change of color from yellow to blue. Different types of samples have been analyzed using these modified strains and compared to standardized methods. The approach showed to be the fastest available tool for coliphage enumeration, detecting up to 1 somatic or F-specific coliphage in 1:30 to 4:00 hours in raw sewage, river water, sludge or mussels samples. This strategy has also been applied to microplates or to large sample volumes and allows qualitative or quantitative analyses.

## **SESSION 6 – WATER TREATMENT AND DISINFECTION**

### **38      From rainwater to a hot shower: challenge testing of a domestic rainwater to hot water treatment system**

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As water shortages and contamination occur more often around the world, the need to find alternative sources of water is on the rise. Whilst widely accepted as an alternative source for irrigation and toilet flushing purposes, highly variable quantity and quality limits the use of rainwater as an additional source of hot water in households.

The systems tested are composed of a filtration device, a UV system and a heat-pump system. A series of challenge tests were conducted on each component and on the full systems in triplicates using semi-synthetic rainwater, various flow and microbe levels over a simulated usage period of one year. *E. coli*, enterococci, *Campylobacter*, *Salmonella* and *MS2 bacteriophage* were tested. The validated dose delivered by the domestic ultraviolet (UV) disinfection unit was also investigated.

The UV systems provided 70% of the treatment of all microbes. The remainders were inactivated through the heat-pump system providing correct retention time and temperature conditions were achieved. MS2 treatment showed a significant linear relationship with the temperature of the water at

the top of the heat pumps ( $Rs = 0.72$ ,  $p < 0.001$ ). Overall, the combined systems managed to treat up to 4.5 log of all faecal indicator bacteria for the majority of the tested conditions. The UV validation testing showed that under the most favourable conditions (10L/min, 90% UVT), the UV systems were validated for >4 log reductions for both *Cryptosporidium* and *Giardia*. During the most challenging conditions (20L/min, 60% UVT), none of the triplicate could be validated to more than one log reduction even with a new UV lamp.

Both the challenge test and the UV validation work showed the potential of this system to reduce health risks and helped develop guidelines for the usage of such systems to provide an alternative source of hot water in domestic settings.

## **39 Phage based Approach for Waste Water Treatment**

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Bhavan's College

Water security to protect human lives and support sustainable development is one of the greatest global challenges of this century. Waterborne bacterial pathogens in wastewater remains an important public health concern, not only because of the environmental damage, but also due to the high cost of disinfecting wastewater by using physical and chemical methods in treatment plants. Bacteriophages are proposed as an alternative biological method for wastewater treatment, as, the use of chemical disinfectants is curtailed by regulations aimed at minimizing the formation of harmful disinfection by products. Bacteriophages (phages), are viruses that exclusively infect bacteria, and are the most abundant (and perhaps the most underutilized) biological resource on earth, and hold great promise for targeting problematic bacteria. The present study evaluated the presence of pathogenic organisms like *E.coli*, *Salmonella*, *Shigella*, *Citrobacter* etc. from 7 STP samples of Mumbai City, and the enrichment and isolation of specific lytic bacteriophages against these target bacteria obtained from secondary treated waste water. Lytic phages against standard bacterial strains as well as against those isolated from sewage were successfully enriched and isolated by liquid enrichment techniques and assayed by double-agar overlay method. Host Range studies helped to further evaluate the phages on the basis of their lytic ability. *Bacteriophage P5*, having a host range against a wide variety of environmentally isolated antibiotic strains of the family *Enterobacteriaceae* has been isolated and its ultrastructure further studied by TEM. TEM analyses have classified the phage as member of the Order *Caudovirales* and Family *Myoviridae*. Based on its ultrastructure studies, host range, MOI and EOP, a consortia of potent phages has been prepared which has a 30-50% efficiency in reducing bacterial pathogens of the *Enterobacteriaceae* family and thus can be used as a promising biocontrol agent supplementing the traditional waste water treatment procedures.

## **40 Enteric virus contamination in hospital wastewater of the Kathmandu Valley, Nepal**

Sarmila Tandukar (A), Jeevan B. Sherchand (B), Bikash Malla (A), Rajani Ghaju Shrestha (C), Ocean Thakali (A), Eiji Haramoto (A)

University of Yamanashi, (A); Tribhuvan University, (B); Osaka University, (C)

Hospital wastewater (HWW) represents a major source of spreading pathogenic microorganisms in the aquatic environment. In Nepal, HWW is considered to be domestic wastewater and discharged directly into the municipal sewer network without any pretreatment. Therefore, the aim of this study was to assess the environmental contamination by viruses responsible for acute gastroenteritis. A total of 17 HWW samples were collected from 3 different cities of the Kathmandu Valley (Kathmandu ( $n = 5$ ), Lalitpur ( $n = 5$ ), and Bhaktapur ( $n = 5$ )) in 2018. All HWW samples collected from 15 hospitals had no facilities of wastewater treatment plants, while from a hospital comprising a treatment plant facility of constructed wetland, both influent and effluent samples were collected. The presence of eight enteric viruses (human adenoviruses (HuAdVs), JC and BK polyomaviruses (JCPyVs and BKPyVs), Aichi virus 1, cosavirus, enteroviruses, group A rotaviruses, and noroviruses of genogroup I (NoVs-

GI) along with plant viruses (pepper mild mottle virus and tobacco mosaic virus) was determined by using quantitative PCR. Plant viruses were found positive in all tested samples, whereas HuAdVs, JCPyVs, and BKPyVs were detected with same positive ratios of 60%. Enteroviruses and NoVs-GIs were the most frequently detected enteric viruses, which were present in 100% samples, in Kathmandu and Bhaktapur. The highest mean concentration was observed for cosavirus ( $6.5 \pm 1.9$  log copies/L) and enteroviruses ( $6.5 \pm 1.2$  log copies/L) in Kathmandu and Bhaktapur, respectively. Similarly, cosavirus (60%) was found the most prevalent enteric virus in Lalitpur. The log reduction value was observed lower than 1.5 for all tested viruses, suggesting that the existing treatment plant had low reduction efficiency. Data on huge number of viral detection in effluents of HWWs confirmed the potential for environmental contamination by viruses and data could be useful to establish standard policies on HWW management.

## SESSION 6 – WATER REUSE AND HEALTH CONCERNS

### 41 Reductions of infectious adenoviruses and reoviruses in conventional and advanced water reclamation systems for reuse are inadequate to meet performance targets

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University of North Carolina

Reclaimed wastewater for reuse requires consideration of its microbial quality. Human enteric virus pathogens, such as adenoviruses and reoviruses found in wastewater are of health concern and are culturable in mammalian cells. Cell cultures are used typically detect the presence and concentrations of infectious viruses in environmental samples, but they often require long incubation periods for virus replication and visible signs of infection as cytopathogenic effects (CPE); some viruses produce no CPE. Molecular methods, such as polymerase chain reaction (PCR), allow direct, sensitive and specific detection of human enteric viruses in the environment by amplifying target nucleic acids but do not distinguish between infectious viruses and genes from non-infectious viruses. Integrated cell culture plus RT-qPCR (ICC-PCR) provides sensitive and specific detection of culturable, infectious viruses even in the absence of CPE. We determined reductions in concentrations of infectious human adenoviruses and reoviruses by ICC-PCR in conventionally treated wastewater and in a pilot multi-barrier reclaimed water system using non-membrane treatment processes. There were significant reductions of infectious viruses between initial raw sewage influent and final reclaimed water effluents of the multibarrier reclaimed water treatment train and the conventional wastewater treatment system. However, reclaimed water virus reductions were similar to those of the conventional wastewater treatment train and did not exceed  $3 \log_{10}$ . The observed  $\log_{10}$  virus reductions do not meet virus reduction performance targets of the State of California, the State of North Carolina or the World Health Organization guidelines for agricultural irrigation or potable reuse. Therefore, improved treatments are needed.

### 42 Quantitative Microbial Risk Assessment of Adenovirus and Rotavirus in the Reclaimed Drinking Water Treated via Short Retention Time of Soil Aquifer Treatment

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Mahidol University, National Institute of Public Health, Kyoto University

Reclaimed water is becoming the worldwide interest as an alternative for the water scarcity solution. Soil aquifer treatment (SAT) is an alternative natural treatment system for various contaminant removal which is low cost and eco-friendly. Typically, it requires a long retention time ( $\geq 6$  months) to ensure the efficient removal of pathogens. However, this is not applicable in urban areas where there is a high demand for readily available water. Researches on short retention time ( $< 6$  months) of SAT has been conducted to ensure whether several chemicals and microorganisms could be removed into the

acceptable level. Nevertheless, the capability of short retention time SAT for human enteric virus removal is not yet known. Thus, the objective of this study aims to evaluate the virological risk assessment of adenovirus and rotavirus in treated wastewater through 1-month retention time SAT. Secondary treated wastewater from Toba Wastewater Treatment Plant, Kyoto, Japan was fed into the SAT column. Adenovirus and rotavirus were detected by qPCR and integrated cell culture-PCR (ICC-PCR). The virological risk was evaluated by Quantitative Microbial Risk Assessment (QMRA) based on the data for infectious viruses and its exposure data. The results showed that the SAT could inactivate the viruses in the range of 1.28-2.03 and 0.43-0.96 log<sub>10</sub> for infectious adenovirus and rotavirus, respectively. Moreover, it exhibited that a virus inactivation mechanism could occur in the SAT. However, the yearly risks of infection via Monte-Carlo simulation suggested that these viruses in the reclaimed water treated via the SAT have not completely met the acceptable level (0.0001 per person per year). Therefore, further treatments, e.g., chlorination and ozonation, are required to achieve more than 8 log<sub>10</sub> removal for potable use of SAT effluent.

#### **43 Seasonal variations in the concentration of pathogenic and indicator microorganisms in raw and treated wastewater**

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**Introduction:** Although wastewater reuse presents numerous potential benefits, the presence of pathogens poses risks to public health. As traditional faecal indicator bacteria (FIB) fail to indicate the removal of viral pathogens in wastewater treatment systems, novel indicators such as bacteriophages have been proposed. It has been reported that concentrations of viruses in wastewater may vary seasonally.

**Aim:** This study aimed at identifying seasonal variations of the concentrations of FIB, phages and viral pathogens in raw and treated wastewater.

**Methods:** Activated sludge (AS) and trickling filter (TF) systems in England had their raw wastewater (RW) and secondary effluent (Sec.Eff) monitored from June 2013 to May 2014 (no. samples=192) for the following organisms: faecal coliforms (FE) and intestinal enterococci (IE) – membrane filtration; somatic coliphages (SOMPH), F-RNA coliphages (F-RNAPH) and phages infecting *B. fragilis* (Bf124PH) – double-layer agar; and human adenovirus (HuAdv) – qRT-PCR. The following seasonal definitions were considered: Spring (March-May), Summer (June-August), Autumn (September-November) and Winter (December-February). Comparisons were performed using ANOVA ( $\alpha=0.05$ ).

**Results:** RW from both AS and TF systems presented levels of FC (6.6-6.7 log<sub>10</sub> cfu.100mL<sup>-1</sup>) significantly higher than IE (5.8 log<sub>10</sub> cfu.100mL<sup>-1</sup>) and SOMPH (5.9-6.1 log<sub>10</sub> pfu.100mL<sup>-1</sup>), followed by HuAdv (4.4-4.5 log<sub>10</sub> copies.100mL<sup>-1</sup>), and then Bf124PH and F-RNAPH (3.5-3.8 and 3.2-3.3 log<sub>10</sub> pfu.100mL<sup>-1</sup>, respectively). AS systems were significantly more efficient than TF systems. FIB and phages' concentrations were significantly higher in Summer and Autumn periods compared to Winter and Spring in both RW and Sec.Eff and in both AS and TF systems; exceptions were Bf124PH in AS RW samples, FC and IE in AS Sec.Eff samples, and IE and Bf124PH in TF Sec.Eff samples. No significant differences were observed for the levels of HuAdv in RW and Sec.Eff samples in AS and TF systems. Annual and seasonal characterization of wastewater may reduce risks to public health associated with wastewater reuse.

#### **44 Drip irrigation with treated wastewater: sanitary issues of biofouling**

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Drip irrigation techniques provide water-saving irrigation solutions that deliver water in controlled quantities for the plant's optimal growth. Nonetheless, biofilm development in these systems fed with

treated wastewater induces biofouling and reduces the benefit of this system. Several experiments were performed in order to characterize the factors affecting biofouling in these systems. In the first study, the influence of dripper hydraulic parameters on biofilm development and on the presence of bacterial genera of health interest was tested. Three types of drippers with different hydraulic parameters and flow rates (1, 2 and 4 L.h<sup>-1</sup>) have been installed on irrigation pipes supplied with lagoon-treated wastewater for 3-4 months. The biofilm development was followed by Optical Coherence Tomography. Bacterial communities of dripper biofilms were characterized by 16S rDNA high throughput sequencing over time. The biofilm development was higher in the 1 L.h<sup>-1</sup> drippers. The structure of the bacterial communities in the dripper biofilms and the abundance of genera associated with opportunistic pathogens (*Legionella*, *Acinetobacter*, *Aeromonas*, *Pseudomonas*, *Mycobacterium* or *Stenotrophomonas*) were different according to the dripper type, which could be explained by hydraulic flow changes. Moreover, the abundance of some of these genera (*Acinetobacter*, *Aeromonas*) in dripper biofilms decreases over time while the bacterial diversity increases. In a second experiment, the effect of treatments (water purge and chlorination) to limit the biofilm development were studied for 1 L.h<sup>-1</sup> drippers. The dripper biofilm development was slower for the chlorinated irrigation pipes. The impact of the treatment on the bacterial communities and on the removal of genera containing opportunistic species will be characterized (analyses in progress).

## SESSION 7 – RECREATIONAL AND SURFACE WATER

### 45      **The Freshwater Microbiological Sciences Review: a proposal to revisit New Zealand's recreational water quality guidelines.**

Sarah Coxon, Elaine Moriarty, Brent Gilpin

Institute of Environmental Science and Research

The degradation of waterways by faecal contamination poses a health risk to people using the water for recreational activities (e.g. swimming), and is an issue of high public interest. In New Zealand, the 2003 Microbiological Water Quality Guidelines for Freshwater Recreation incorporate a risk-based Annapolis approach, with the measurement of *E. coli*. The Guidelines are somewhat unique in that limits are based on a quantitative microbial risk assessment (QMRA) for campylobacteriosis, rather than epidemiological studies. The QMRA was derived from the findings of a comprehensive nationwide survey of microbial water quality.

An essential underpinning of the Guidelines is a robust understanding of the relationship between the pathogens that may be present in the water and the indicator organism(s) used to monitor water quality. Since the microbial survey was undertaken in 1998-99, there have been significant advances in laboratory methods that allow for more sensitive and specific detection of microorganisms, improved characterisation of pathogen virulence, and establishment of faecal source. These advances allow for more accurate assessment of health risk. There have also been significant changes in land use patterns and management practices around New Zealand, which may affect the particular microorganisms entering waterways, their survival in the environment, and/or the indicator-pathogen relationship.

The costs associated with monitoring, managing and improving recreational water quality are estimated to exceed NZ\$200 million annually. It is therefore imperative that the Guidelines are based on sound and up-to-date science. We have designed a study that accounts for developments over the past 20 years, and could support the review of New Zealand's current freshwater Guidelines. It includes a list of candidate sampling sites (and the criteria by which they were selected); discussion of the pathogens and indicator organisms that should be monitored and by which analytical procedures; and the QMRA design and expression of health risk.

**46**

## **Assessment of Dublin Bay bathing water quality: A One Health approach**

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Bathing water quality is critical for public health, economic marine activities and is strongly influenced by pollutants and pathogens from urban and industrial activities as well as weather conditions. Water quality can vary significantly throughout a day, especially on beaches strongly affected by the tide. Current monitoring protocols may not adequately reflect this variability in bathing water quality. The aim of this project was to assess the spatial and temporal variability as well as the source of faecal pollution in Dublin Bay within a One Health approach.

Deep water samples from Dublin Bay were sampled during neap and spring tide and water samples from the seashore from two strands were taken every 30 min over a tidal cycle. In addition, samples were collected along a 2 km perpendicular transect during high tide from the shore. Faecal pollution indicators including somatic coliphages were determined and molecular microbial source tracking analyses were conducted.

Analysis of the faecal indicators demonstrated that the samples collected in the deep Dublin Bay area were found to be of excellent quality. However, the human marker was identified in 80% of the samples and the gull marker in 20%. Faecal indicator levels from nearshore water varied by one order of magnitude during the tide cycle and decreased with the distance from the seashore. These results suggest that bathing water problems originate from nearshore sources. In addition, significant differences were found for enterococci and *E. coli* along a day. These findings suggest a diurnal pattern driven by solar irradiance; therefore, the classification of water quality could be affected by sampling time. This study will be of interest to regulatory agencies and public health bodies as it provides scientific knowledge for the management of bathing water with consideration for marine and coastal ecosystems.

**47**

## **Coupling source tracking and QMRA: risks of swimming in beaches contaminated by diffuse pollution**

Lynze Cheung, Muriel Lepesteur-Thompson, Christelle Schang, Rebekah Henry, David McCarthy

Monash University, EPA Victoria

Our understanding of the risks to users of beaches that are fed with non-point, non-human sources of pollution is limited. The majority of recreational epidemiological studies have been done on beaches fed with known sources of human contamination, yet the risks in swimming in beaches that are predominately fed with non-human sources are likely different. In this study, we begin to understand the risks to users of Port Phillip Bay, a local hot spot for more than 2million swimmers each year, through the coupling of QMRA and microbial source tracking. We collected 60 samples from three beaches over the 2017-2018 summer, and analysed these for a suite of indicator organisms. The 95th percentile enterococci concentration across three sites was 370 MPN/100mL, which according to local guidelines, the probability of illness per swimming event is between 5-10%. 16s amplicon sequencing was conducted on these beach samples and, using the SourceTracker tool, their taxonomic profiles were compared to those obtained from local wildlife, human sewage, and human wastewater taken from a variety of treatment plants around the vicinity. On average, 22% of faecal contamination found in the beach water was derived from dogs, 13% from human sewage, 8.8% from seagulls and other waterfowl, 4.3% from chickens, and 1.9% from equines (no other source was detected). Using these percentages, enterococci concentrations found in the beach water were apportioned into these five sources. Enterococci/pathogen ratios, prevalence rates and likely infectious proportions were obtained

for each faecal source using literature datasets. Using this data, we obtained predicted concentrations of *Campylobacter*, *Salmonella*, *Giardia*, *Cryptosporidium*, and noroviruses in the beach water samples. Following the WHO's harmonized QMRA framework, we determined that the probability of illness per swimming event is, on average, 3.7%. The full presentation will present the comparison to our QMRA when conducted with monitored pathogens.

#### **48           Health risks of swimming in beaches contaminated by non-point pollution sources: a case study of three Melbourne beaches**

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Current Australian guidelines and policies for managing recreational risks are derived from epidemiological studies conducted on beaches that were fed with human wastewater and that had high oceanic exchanges. This study had an underlying hypothesis that managing health risks is unique for bay beaches primarily fed by diffuse, non-point, non-human sources of pollution. To test this hypothesis, we first conducted a meta-analysis of recreational epidemiological studies, revealing that both contamination source (human vs. non-human) and water body type (oceanic vs. embayment) significantly influenced the shape and slope of the relationship between indicator organism concentrations and measured health outcomes. We then conducted a QMRA on three bay beaches, primarily fed with non-human sources of contamination, using data from sixty 60L samples, taken twice per week during the Australian summer of 2017-2018. Our enterococci data, combined with our sanitary survey, was first used to assess the beaches as per our current guidelines, which suggested that the probability of GI illness per primary contact even was between 5-10%. However, our pathogen concentrations were generally low (only 8% samples were above detection for *Campylobacter*, 23% for *Salmonella*, 3% for *Cryptosporidium* and 7% for adenoviruses), and the Monte Carlo QMRA simulations had a 95th percentile probability of illnesses per contact event of 1.1% for primary contact activities. These are nearly one order of magnitude lower than those predicted using the guidelines. These QMRA results compared well with our log-logistic models developed in the epidemiological meta-analysis. Our next task used source tracking to determine the likely origin of the measured enterococci; the results demonstrated that, on average, 13% of the enterococci was from a human origin. While this provides some evidence toward understanding risks and indicator relationships in non-oceanic beaches fed with non-point sources of pollution, epidemiological studies are required to validate these findings.

#### **49           Protecting drinking water quality while enabling recreational access: new tools to quantify water treatment risk**

Brooke A. Swaffer, Jeffrey Newman, Rob Daly

South Australia Water Corporation

As global populations become increasingly urbanised, there is mounting public pressure to open traditionally closed areas for land and water-based recreational activities. At first glance, the natural setting of drinking water reservoirs offers an attractive option, due to their often pristine condition and proximity to major metropolitan centres.

Considering links between recreation and source water contamination are well established, the major challenge for drinking water providers is designing a regime that can deliver community-level benefits whilst protecting water quality.

To facilitate decisions in this regard, South Australia Water Corporation' scientists have developed an innovative tool which can be used to inform evidenced-based decisions on recreational activities that would be unlikely to impact product water quality, or compliance with legislated obligations. We will present the tool and underlying framework, which combines the latest available scientific data, three-dimensional hydrodynamic water quality modelling, operational protocols, and quantitative microbial risk assessment methods as well as recognise health-based water quality thresholds to predict risk to drinking water treatment processes, and ultimately, product water safety.

The tool is unique as it does not rely on static assessments nor deliver finite outputs, but is rather interactive and instantaneous. It can rapidly re-assess different scenarios and provide an immediate, but simple, prediction of drinking water risk. This agility has already proved to be a successful element in bridging the communication gap between water quality scientists and policy decision makers, ultimately providing them with adequate information to allow decisions based on a renewed understanding of recreational access impacts .

By doing so, SA Water has set a benchmark for the water industry by developing a decision-support framework which will be relevant to any utility interested in quantifying water quality risk from recreational access in their catchments or reservoir storages.

**50                  Detection, quantification and land use mapping of *Cryptosporidium* sp. and *Giardia* sp.in surface water supplies at the state of São Paulo, Brazil**

Bruna Suellen Breternitz, Denise Piccirillo Barbosa da Veiga, Maria Tereza Pepe Razzolini and Adelaide Cassia Nardocci

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The association between the quantification and the distribution of *Cryptosporidium* and *Giardia* in the environment, and the environmental characterization of watersheds and hydrological models allow the identification of environmental circulation patterns of the parasites. The objective of this research was to relate the occurrence and circulation of these parasites to the occupation of the watershed lands, which are mainly located in rural areas, where there is no sanitary sewage, and livestock is an important economic activity. The study involved 11 municipalities in the state of São Paulo, located in southeastern Brazil. Sampling was carried out at the catchment sites of each water supply on a monthly basis, starting in December 2014 and lasting until November 2015, totaling 128 samples. Protozoan were quantified according to the 1623.1 US.EPA Method (USEPA, 2012). For delimitation of the drainage area of the water catchment area of each municipality, the hydrographic network was extracted from the hydrology tool of ArcGIS software. For the classification of land use/land cover, RapidEye satellite images, dated October 2014 and made available by the Brazilian Ministry of the Environment, were submitted to the Supervised Maximum Likelihood Classification method. Google Earth Pro images were used in order to facilitate the visual interpretation process, and field work was used in order to quantify agricultural land use within the catchment area. The frequency of occurrence of these pathogens and the high concentrations were evidenced in the municipality with the largest urban area (16.2%) and intense livestock activity (39%) near the catchment site. The municipality that showed the lowest frequency of occurrence, presented the smallest urban area (0.87%) and absence of livestock activity near the catchment site. The high concentration of pathogens suggests a correlation between the impact on water supply networks and river basin degradation caused by urban activity and livestock.

## **51 Microbial water quality of surface and floodplain waters after Hurricane Florence**

Angela Harris, Ryan Emanuel, Sophia Kathariou, Jeffrey Niedermeyer, Natalie Nelson, William Miller, Theo Jass

North Carolina State University, United States Department of Agriculture

North Carolina's Atlantic Coastal Plain, home to many impoverished and minority communities as well as concentrated animal feeding operations (hog and poultry), experienced extreme regional flooding following rainfall from Hurricane Florence (2018). The juxtaposition of extreme flood risks, ecological and health hazards, and communities that are both socially vulnerable and land-dependent creates a volatile situation during storms. The aim of this study was to characterize the biological quality of flood water and surface water after the hurricane in 4 impacted watersheds in Eastern North Carolina. Water samples were collected from streams, floodplains, and isolated ephemeral water bodies at 46 different sites within two weeks (Phase 1) of the hurricane making landfall, as well as one month later (Phase 2). Using standard methods, we enumerated *E. coli* in the water samples, as well as human (HF183 Taqman) and swine (Pig-2-Bac) specific molecular microbial source tracking (MST) targets. Prevalence of *Listeria monocytogenes*, *Salmonella*, *Campylobacter*, and *Arcobacter* was determined via selective enrichment of the samples. Thirty percent of the samples were above the most probable number of *E. coli* per 100mL. Phase 1 and Phase 2 samples had similar levels of *E. coli* (Wilcoxon signed rank test,  $p>0.05$ ), and during Phase 2, flowing stream samples had higher concentrations of *E. coli* than floodplain samples (Mann-Whitney test,  $p<0.05$ ) (differences between sample type not observed in Phase 1). Samples were positive for *L. monocytogenes* (1%), other *Listeria spp.* (18%), *Salmonella* (5%), *Campylobacter jejuni* (1%), and *Arcobacter* (73%). 67 novel sequence types for *Arcobacter* were identified via multilocus sequence typing. MST results are forthcoming. Future sampling phases will afford the opportunity to gather data on this system during non-flooded conditions. Together these data will help inform recommendations for emergency response and waste management strategies to protect human health during extreme floods.

## **SESSION 8 – METHODS AND SURVEILLANCE**

### **52 “Know your catchment”: using online near-real time monitoring of *E. coli* to track pollution sources of fecal pathogens in a drinking water supply under de facto wastewater reuse conditions**

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Past waterborne outbreaks revealed the vulnerability of drinking water resources to intermittent fecal pollution peak episodes. Whereas compliance monitoring of source water is currently not designed to accurately identify hazardous events, developments in online microbial monitoring technologies is expected to improve current monitoring frameworks.

We conducted online near-real time monitoring of  $\beta$ -D-glucuronidase (GLUC) activity for >2.5 years at the intake of an urban drinking water treatment plant (DWTP) in the Greater Montreal area. Parallel routine and event-based monitoring of culturable *E. coli* and protozoan pathogens were performed at the intake.

GLUC activity fluctuations ranged over several time scales, from seasonal to hourly. Most peak contamination events occurred between late fall and early spring following intense precipitation and/or snowmelt.

We found a significant linear correlation between GLUC activity and both culturable *E. coli* and *Giardia* and currently develop a negative binomial regression model to accurately assess the relationships between GLUC activity and protozoan pathogens using additional data.

In absence of rainfall, GLUC activity displayed recurrent daily fluctuations, indicating the impact of treated wastewater effluents. The hydraulic connection between the intake and a 6-km upstream Water Resource Recovery Facility (WRRF) was demonstrated by cross-correlation analysis of time series from both sites.

These original findings are complemented by those obtained at a downstream DWTP intake also impacted by a local WRRF and combined sewer overflow discharges within the same catchment.

Altogether, our data demonstrate that online GLUC activity can provide crucial information on catchment microbial dynamics under *de facto* wastewater reuse settings, by (i) characterizing critical periods of microbial challenge otherwise missed by routine monitoring, (ii) identifying upstream fecal pollution sources and (iii) providing a framework for targeted pathogens sampling to inform about microbial risk. Ultimately, this knowledge should help prioritize source mitigation actions towards protection of drinking water supplies and public health.

### **53 Evaluation of the novel substrate RUG™ for the detection of *Escherichia coli* in water from temperate (Zurich, Switzerland) and tropical (Bushenyi, Uganda) field sites**

Franziska Genter, Sara J. Marks, Guillaume Clair-Caliot, Deo S. Mugume, Richard B. Johnston, Robert E. S. Bain and Timothy R. Julian

Eawag Swiss Federal Institute of Aquatic Science and Technology Duebendorf Switzerland, ETH Swiss Federal Institute of Technology Zurich Switzerland, NWSC National Water and Sewerage Corporation Bushenyi Uganda, WHO World Health Organization Geneva Switzerland, UNICEF New York USA

Direct testing of water quality to promote drinking water safety contributes to the Sustainable Development Goals, which call for universal access to safely-managed drinking water services by 2030. Enzyme-substrate tests offer a potentially simple and reliable approach for the detection and quantification of fecal indicator bacteria, including *Escherichia coli* (*E. coli*). The novel aquatest (AT) based on resorufin- $\beta$ -D-glucuronide methyl ester (RUG™) (AT-RUG) is an enzyme substrate test that overcomes several drawbacks of other established tests. In this study, AT-RUG was used to detect and quantify *E. coli* in water from temperate (Zurich, Switzerland) and tropical (Bushenyi, Uganda) regions. Quantitative results of AT-RUG were compared with IDEXX Colilert-18® (C-18), m-TEC and m-ColiBlue24®. In temperate waters, AT-RUG was found to be as sensitive as m-TEC (97.0 %) and C-18 (98.5 %) and showed strong agreement with the reference methods. The false-positive rate for *E. coli* detection in temperate waters using AT-RUG was 6 %. AT-RUG performed well at incubation temperatures of 37 °C and 45 °C, but not at 24 °C. In tropical waters, the sensitivity of AT-RUG was 94.1 % compared to m-ColiBlue24®. AT-RUG detected significantly more *E. coli* than m-ColiBlue24®, suggesting it is a more conservative estimate. At both field sites, AT-RUG was able to effectively indicate categorical concentrations of *E. coli* in water samples indicating the level of risks of fecal contamination of water supplies. This study indicates that AT-RUG is a reliable and accurate medium for the detection and quantification of *E. coli* in temperate and tropical waters.

### **54 Distribution of *E. coli* Pathotypes Along an Urban-Rural Gradient in Ecuador**

Lorena Montero, Shanon Smith, Maritza Paez, Estefanía Ortega, Angela Pena-Gonzalez, María Juliana Soto-Girón, Janet K. Hatt, Xavier Sánchez, Edison Puebla, Pablo Endara, William Cevallos, Konstantinos T. Konstantinidis, Gabriel Trueba, Karen Levy

Emory University, Georgia Institute of Technology, Universidad San Francisco de Quito, Universidad Central del Ecuador

**Background:** Diarrhea is caused by multiple pathogens. Previous studies have captured between- but

not within-country heterogeneities in enteropathogen prevalence and severity. Focusing on how enteric infection rates differ within the same country avoids confounding factors such as different cultural context or diet. **Methods:** We conducted a case-control study of diarrhea to understand how rates and outcomes of enteropathogen infection vary across an urban-rural gradient in four sites in northern Ecuador. **Results:** We found variability by site in enteropathogen prevalence and outcomes of infection. Any pathogenic *E. coli* infection (OR = 1.46, 95% CI 1.06-2.02), coinfections (OR = 2.76, 95% CI 1.07-7.13), diffuse adherent *E. coli* (DAEC; adjusted odds ratio (aOR)= 2.10, 95% CI 1.29-3.46), enteroinvasive *E. coli* (EIEC) (aOR = 12.06, 95% CI 2.25-223.3), and rotavirus (aOR = 32.24, 95% CI 9.56-208.7) were significantly associated with diarrhea. DAEC was the most common pathotype, and was more common, and more associated with disease, in urban areas. Enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) were more common in rural areas. ETEC was only associated with diarrhea in one site. Phylogenetic analysis revealed that associations with disease were not driven by any single clonal complex. Higher levels of antibiotic resistance occurred in rural areas. **Conclusions:** Enteropathogen prevalence, virulence, and antibiotic resistance patterns vary substantially by site within a country. DAEC is a potentially important pathogen, especially in urban areas, and should be included in future studies. Control of pathogens in urban areas could be a strategy to control downstream effects on disease in rural areas.

## 55 Experiences from full-scale applications of flow cytometry for microbial monitoring of drinking water in Austria

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Over the last decades flow cytometry has proven itself as a robust method of rapid detection and analysis of cells in medical diagnostics, biotechnology and research. More than a decade ago researchers from the Swiss Eawag successfully began expanding the field of application into drinking water monitoring with a number of Swiss and Dutch drinking-water suppliers routinely using the method nowadays. The latter step still has to be made in Austria, but nonetheless considerable research efforts including extended measurement campaigns led to new insights into microbial dynamics of drinking water systems as well as a growing awareness of new methods in the drinking water community.

The here presented works aims to sum up recent examples of full-scale application of flow cytometry for monitoring drinking water supply systems in Austria that comprise a comparable frequency and amount of samples as the routine bacteriological examination. Following the water's way from resource to the consumers tap fine-grained as well as major microbial changes are detected. These changes consist in shifts of total cell counts, intact cell counts and the proportion of cells with high nucleic acid content being indicative for regrowth processes. Whereas most of the investigated water resources have stable characteristics over time, some exhibit considerable variations even affecting intact cell counts after chlorination. In multiple cases storage tanks could be identified as sites, where named alterations occur. Persistent changes appear as distinct microbial clusters or patterns in the course of flow cytometric measurements and thus can be tracked on subsequent sampling points.

Flow cytometry has proven its capabilities for a sensitive microbial monitoring and thus bears the favorable prospect of an in-depth description of harmless or desirable as well as problematic biological processes. Its full potential for the optimization of drinking water supply systems is yet to be uncovered.

**56 CAFÉ: A Sensitive, Robust and Cost-Effective Filtration Method to Detect Poliovirus and Other Enteroviruses from Residual Waters**

Hanen Belgarsi-Wright, Angela Coulliette-Salmond, Kimberly Wong, Shelina Moonsamy, Heleen du Plessis, Ma. Anne-Lesley Valencia, Ratana Tacharoenmuang, Apradee Isarangkul Na Ayutthaya, Ratigorn Guntapong, Lea Apostol, and Everardo Vega

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Acute flaccid paralysis (AFP) surveillance has been responsible for identifying polio since the inception of the Global Poliovirus Eradication Initiative (GPEI) in 1988. Environmental surveillance (ES) is an important adjunct to AFP surveillance and along with AFP cases and contacts are referred to one of the 146 laboratories in the Global Polio Laboratory Network. WHO-supported laboratories use the two-phase separation method, due to its simplicity and effectiveness, to concentrate 500 mL of sewage, but alternative methods that are simple, cost-effective, and not tied to proprietary suppliers are desirable. The CAFÉ (Concentration and Filtration Elution) method was developed from existing filtration methods to handle any type of sewage or residual waters. The CAFÉ method uses a stainless steel coffee press with a cellulose filter to collect heavy sediment followed by a series of two filtration steps using a 5 µM and 0.45 µM filters. Trapped viruses on filters are then extracted and assayed using the GPLN poliovirus isolation procedure. At \$10-20 per sample, CAFÉ is cost-effective and all equipment and reagents are available on the common market around the world. To measure the effectiveness of CAFÉ compared to the two-phase method, we designed a parallel study involving six countries, Guatemala, Haiti, Thailand, Papua New Guinea, Zambia, and the Philippines, to account for regional and sample-to-sample variability. Prospective samples from each site were divided into two equal 500 mL aliquots and processed by the two methods, with no other additional concentration or manipulation. Preliminary results of 119 parallel-tested samples show that CAFÉ method is more sensitive than two-phase for non-polio enteroviruses [86 detected CAFÉ vs 65 by two-phase:  $P < 0.001$ ] and polioviruses [17 detected by CAFÉ vs 4 by two-phase:  $P < 0.001$ ]. The CAFÉ method is a robust, sensitive, and cost-effective method for isolating enteroviruses from residual waters.

**57 Norovirus accumulation in wild estuarine bivalves as an indicator of gastroenteritis epidemics: Comparison between oysters and clams (*Nuttallia japonica*)**

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Previous studies reported heavy contamination of oysters with Norovirus (NoV) excreted by patients of gastroenteritis in the epidemic season, suggesting that monitoring of NoV in oysters is useful for surveillance of epidemic of viral gastroenteritis. Other estuarine bivalves can also be used for the surveillance if they accumulate NoV effectively. This study aimed at comparative monitoring of NoV in oysters and clams (*Nuttallia japonica*) as common estuarine bivalves living in rocky and sandy zones, respectively. From October 2017 to November 2018, twenty samples of each bivalve were collected monthly from an estuary in Sendai, Japan, except the epidemic season (November to March) with twice-a-month sampling. Digestive tissues (DT) isolated from four bivalve samples were composited and NoV-GI and GII in the composites were quantitatively detected by qPCR analysis. The result of monitoring demonstrated that clams accumulated NoV comparably to oysters at the concentration level of  $10^2$  to  $10^4$  copies/g-DT. The NoV concentrations increased in 2018 epidemic season, while such a seasonality was not found in the previous year. In the epidemic season, the NoV-GII concentrations in both bivalves showed significantly positive correlations with the number of gastroenteritis patients reported in Sendai city one and three weeks before the sampling of bivalves, respectively. Clams living in sand may have taken a longer time to accumulate NoV than oysters

directly impacted by river water receiving treated wastewater from the city. The relationship between bivalve contamination and gastroenteritis epidemics was not clear for NoV-GI since this genogroup is rarely detected from the gastroenteritis patients. Moreover, the pyrosequencing analysis for NoV-GII identified GII.2, GII.4, GII.17 and GII.21 in oyster samples, three of which were commonly detected in sewage from Sendai city in the same period. On the other hand, no genotypes were obtained from clam samples probably due to inhibitory effects of clam-origin materials.

**58 Viable but nonculturable (VBNC) *Legionella pneumophila* induced at high temperatures: viability and infectivity patterns**

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Temperature management is commonly used to prevent the proliferation or reduce the concentrations of *Legionella* in engineered water systems (EWS). In response to such stress, culturable *Legionella* populations can switch into a viable but nonculturable (VBNC) state. This state is seen as a survival strategy, but it is still unclear how relevant VBNC *Legionella* cells are for human health. Here, we investigated the stress response patterns and transitions of the bacteria to the VBNC state at 55°C, 60°C and 70°C on two *L. pneumophila* strains for >80 days using a combination of cell-based viability indicators. Complete loss of culturability at 55°C, 60°C and 70°C occurred after 3-8 hours, 60 min and <2 min, respectively. In contrast, *L. pneumophila* strains required 9 days at 55°C, 8 hours at 60°C and 20 min at 70°C to achieve a 2 log reduction in cells with intact membranes and high esterase activity; a 4 log reduction was achieved only after 150, 8-15 and 1-4 days, respectively. In parallel, the infectivity patterns of the two strains towards amoebae and THP-1 cells were assessed. Nonculturable *L. pneumophila* cells infected amoebae and THP-1 cells for at least 85 days at 55°C and 60°C and for up to 8 days at 70°C. However, they did so with reduced efficiency requiring prolonged co-incubation times with the hosts and higher *Legionella* concentrations in comparison to culturable cells. Consequently, infection in amoebae of thermally induced VBNC *L. pneumophila* with lowered virulence can be expected in EWS. For the first time we show that thermally induced VBNC *L. pneumophila* cells can directly infect THP-1 cells. Whether these cells can infect humans and cause disease remains an open question. Although the gold standard method culture-based cannot detect VBNC *Legionella*, it provides important information about the most virulent bacterial subpopulations.

**59 Enrichment of free-living amoebae in biofilms developed at upper water levels in drinking water storage towers: an inter- and intra-seasonal study.**

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Free-living amoebae (FLA) are ubiquitous protozoan organisms present in various natural and artificial environments, such as drinking water storage towers (DWST). Some FLA, such as *Acanthamoeba* sp., can cause severe infections at ocular or cerebral levels in addition to being potential reservoirs of other pathogens. In this work, the abundance and diversity of FLA was evaluated in two sampling campaigns: one performed over five seasons in three DWST at three

different depths (surface, middle and bottom) in water and biofilm, and one based on the kinetics analysis of biofilm samples collected every two weeks during a 3-month period at the surface and the bottom of a DWST. In the seasonal study, the FLA were detected in each DWST water in densities of ~ 20 to 25 amoebae/L. A seasonal variation of amoeba distribution was observed in water samples, with maximal densities in summer at ~ 30 amoebae L<sup>-1</sup> and minimal densities in winter at ~ 16 amoebae/L. The FLA belonging to the genus *Acanthamoebawere* detected in two spring sampling campaigns, suggesting a possible seasonal appearance of this potentially pathogenic amoeba. Interestingly, a 1 log increase of amoebae density was observed in biofilm samples collected at the surface of all DWST compared to the middle and the bottom ones where FLA were at 0.1-0.2 amoebae/cm<sup>2</sup>. This marked higher amoebae density in biofilm collected in surface samples was confirmed in the kinetics study where an increase of amoebae density, as well as total cell density, and biofilm thickness were observed as a function of time. These phenomena were not observed at the bottom. To our knowledge, this study describes for the first time a marked higher FLA density in biofilms collected at upper water levels in DWST despite the presence of chlorine residual, constituting a potential source of pathogenic micro-organisms.

## **SESSION 9 – QMRA, DECISION ANALYSIS, WATER SAFETY PLANTS AND SURVEILLANCE**

**60**

### **Literature review of pathogen removal treatment for QMRA in a webtool**

Patrick Smeets (A), Alexandra Hockin (A), Mark Morley (A), Wim Hijnen (A, B), Karl Linden (C), Kaitlyn Jeanis (C), William Seites-Rundlet (C), Gertjan Medema (A, D)

(A) KWR Watercycle Research Institute, (B) Evides Water Company, (C) University of Colorado Boulder, (D) Delft University of Technology

Microbial safety of drinking water is of utmost importance for health. In many regions of the world, water shortage leads to direct or indirect reuse of wastewater or use of other unsafe water sources. Quantitative Microbial Risk Assessment (QMRA) can be used to determine which combination of water treatment processes is needed to provide safe water. Assessing treatment efficacy for removing pathogenic microorganisms is a critical element of QMRA. In many cases, the required microbial monitoring data for a site specific assessment is missing and treatment efficacy needs to be estimated based on literature. The WHO GDWQ provides such generic information in Tables 7.7 and 7.8. However, due to the limitations of such a table, sufficient information to apply the findings to the local situation are missing. Accordingly, a webtool was developed that retrieves background information alongside the log removal data obtained in the literature review for table 7.7 to support the legislative QMRA in the Netherlands. The tool allows the interactive selection of conditions by the user in order to provide relevant log removal values from the literature and the publications they were obtained from. Thus, the tool provides a complete overview of current knowledge of treatment efficacy, but also illustrates the knowledge gaps or inconsistent findings in various studies. One important finding is the inadequate reporting of findings in many studies which lack the reporting of some important information. The tool is freely available for use. We will present the tool, how it was developed and the advantages and challenges it provides. We would like to receive feedback on its use and further development through collaboration.

## **61 Norovirus risk assessment: Many subsurface water supplies need to be treated**

Monica B. Emelko; Philip J. Schmidt; Mark A. Borchardt

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Subsurface water supplies often receive less treatment than surface waters: indeed, there are jurisdictions where treatment of some such supplies may not be required at all. Enteric viruses are a potential concern in groundwater under the direct influence of surface water and when groundwater supplies are not chemically disinfected or otherwise treated. Lack of standardized monitoring approaches and consensus on appropriate dose-response relationships has limited the application of quantitative microbial risk assessment (QMRA) to viruses in groundwater.

In this study, norovirus qPCR data from groundwater supplies serving fourteen small communities in Wisconsin are used in a reverse QMRA framework to evaluate desirable levels of treatment. Notably, these supplies are not categorized as being influenced by surface water. Although norovirus genomes were not detected in 91.8% of samples, they were detected with an average of 3.84 genomic equivalent copies (gec) per litre and a maximum of 264 gec/L. A variety of dose-response models, based on both rotavirus and norovirus, were considered in the QMRA. All models fit to the available norovirus dose-response data indicated that a 4-log (99.99%) treatment guideline is warranted. This is consistent with a Health Canada guideline recommending 4-log treatment for viruses in all water supplies. In contrast, the rotavirus dose-response model and manipulated versions of the norovirus dose-response models advocated by WHO/USEPA guidance documents suggest a need for an additional 3-log reduction. This highlights the need for additional scientific consensus on appropriate norovirus dose-response models.

In many cases, a 4-log treatment guideline for enteric viruses in groundwater could easily be achieved through chemical disinfection. Monitoring may demonstrate that some high-quality subsurface water supplies do not require this much treatment, while supplies affected by surface water or subsurface wastewater discharges may require further treatment. Accordingly, standardized quantitative methods and protocols for monitoring enteric viruses in water are needed.

## **62 Understanding the delivery of human-infectious *Cryptosporidium* into reservoirs: informing QMRAs and water treatment requirements**

Brooke A. Swaffer, Hayley Abbott, Brendon King, Leon van der Linden, Paul Monis

South Australia Water Corporation

Treating drinking water safely depends, in part, on the robustness of source water quality risk assessments. However, quantifying the proportion of human-infectious *Cryptosporidium* oocysts, the ultimate determinant of health risk, remains a significant challenge for water utilities world-wide.

We applied over 15,000 source water-derived oocysts to a single format *in vitro* cell culture assay and applied PCR-based molecular techniques to 345 water samples to profile the occurrence, fate, transport and survival of human-infectious *Cryptosporidium*. Importantly, these samples were collected at, or profiled across, the peak of rainfall-runoff hydrographs, intensively characterising episodic events which deliver the highest volume (but lowest quality) water into drinking water reservoirs.

Across 10 locations in southern (temperate) Australia, the annual average infectivity of oocysts reached 18 % during the six-year study; however, most locations averaged less than 5 %. The maximum recorded infectivity fraction within a single rainfall runoff event was 65.4 %, and was dominated by *C. parvum*. Twenty-two *Cryptosporidium* species were identified using PCR-based methods; most commonly *C. parvum*, detected in 25 % of water samples.

Application of the infectivity data into a quantitative microbial risk assessment (QMRA) suggested

that the annualised treatment requirements could be reduced by up to 2.67 Log<sub>10</sub> if measured infectivity data was used, rather than adoption of assumed values, and demonstrated the potential benefit of collating such data for water safety plans.

Despite many methodological advances, water utilities have, until now, struggled to quantify human-infectious oocysts with the degree of certainty required to inform investment decisions. This cell culture assay, and subsequent infectivity data, ultimately benefit drinking water providers by enabling more flexible and accurate source water assessments, potentially avoiding or delaying significant capital expense.

This smart source water monitoring arrangement, coupled with an innovative infectivity assay, is relevant to any provider required to quantify treatment for *Cryptosporidium*-impacted waters.

### **63 Confounding risk factors obscure link between intermittent water supply and gastrointestinal illness in Njoro Town, Kenya**

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Njoro Town is an agro-pastoral community in Kenya with chronic intermittent water supply (IWS) lasting only 4-6 hours a day, 2-3 days weekly. Waterborne illnesses are prevalent (1.7%), typhoid fever (49% of all reported cases of waterborne illness) gastroenteritis (29%) from data at health centre in the township. A microbiological examination of water samples was undertaken to associate IWS with incidences of gastrointestinal illness. Households were selected from a stratified sub-sample of 20 households where gastrointestinal illnesses were reported. Households were matched based on income, location, drinking-water source, presence of Household Water Treatment (HWT). Within 4 - 7 days after a self-report of illness, water samples were obtained from the water sources used, stored water in households and water vendors containers. *Escherichia coli* (EC) was undetected at the source 100% of the time. Median EC densities in high-income households' containers (Median: 40 CFU/100 mL; Range: 0 - 500) with intermittent piped-water on premises were lower compared to low-income households (360; 0 - 520) supplied by water vendors. Vendors distributed water from boreholes (18; 0 - 220) and a nearby stream (900; 350 - 2100) but EC densities increased in vendor-supplied low-income households with borehole water (360; 10 - 520) and stream water (1400; 120 - 3300). Low-income households exposed to contaminated stream water reported (14 - 20 cases/week), high-income households without HWT (4 - 8 cases/week) and boreholes with HWT (0 - 2 cases/week) of gastrointestinal illness. Several confounding risk factors, (poor household hygiene and domesticating livestock within the households), were found 100% in all households, and strongly associated with over 80% of all gastrointestinal illnesses. IWS was observed to be associated with gastrointestinal illness although the link was masked by confounding risk factors. Interventions for improving drinking-water supply to safeguard associated health benefits should provide for uninterrupted access and advocate treatment in households.

**Keywords:**Gastrointestinal illness; confounding risk factors; intermittent water supply (IWS)

**64      Health risk assessment (QMRA and DALYs) related to enteric pathogens in urban flood water – A case study from an urban flood event in Vietnamese Mekong area – Ninh Kieu district, Can Tho city**

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Microbial pathogens in urban flood waters have showed high risk to human health, however, there is lacking of health risk assessment in urban flood waters in low-to-middle income countries (LMICs). To that aim, our study assessed health risk related to enteric pathogens in flood water in Can Tho city – an urban area in Vietnamese Mekong delta. Quantitative microbial risk assessment (QMRA) and Disability-Adjusted Life Years (DALYs) were used in this study. The flood event was in October 2016 with heavy rainfall during high tide period. The exposure population was related to traffic activity – a common practice in case study with four groups: pedestrian children/pedestrian adults/riders/pillion passengers. In order to reduce the uncertainty in estimating exposed population, traffic counting was applied. Flood water was highly polluted with *E. coli* and Rotavirus A during flooding time with  $7E+03$  CFU/mL and  $5E+05$  gc/mL, respectively. The mean infection probability was highest for pedestrian children, following by pedestrian adults, riders, and pillion passengers. DALYs per event for all exposed population was  $7.68E+02$ . DALYs per person per event was  $2.15E-02$  which was much higher than WHO reference DALYs per person per year  $1E-06$  –  $1E-04$ . Rotavirus contributed the most value in DALYs (more than 90%). Riders contributed the highest value in DALYs (84.06%), then pillion passengers (14%), and pedestrians. As our knowlegde, this is the first study that considering both infection probability and DALYs for health risk related to pathogens in flood water. The combination of P-inf and DALYs helps to have better understanding about microbial risk through exposing to urban flood water. During flooding time, by exposing to “too dirty” flood water, urban residents may suffer from waterborne diseases which reduce their healthy life years, especiaaly for riders.

**65      Particle removal during river water treatment with regard to detection of pathogens**

Beate Hambisch, Johannes Ho, Pia Lipp

TZW: DVGW-Technologiezentrum Wasser, Karlsruhe

In some water works in Germany, river water is used as raw water for drinking water treatment. Treatment by rapid sand filtration and slow sand filtration or bank filtration is commonly used in these. Within a research project funded by DVGW, three such river water treatment plants were analyzed for pathogens and indicators for 2 years, allowing quantitative microbial risk assessment (QMRA) for the treated water after soil passage.

As reference pathogens *Cryptosporidium*, *Giardia*, *Campylobacter*, *human Adenovirus* and *Enterovirus* were selected. Different indicators were analyzed and evaluated as indicators for determination of particle removal. The monitoring program was performed over a period of 2 years with monthly measurements. Coliform bacteria were chosen as indicators for the removal of bacteria as well as for parasites, where the smaller bacteria are a worst case scenario. For viral pathogens, somatic coliphages were used as indicators, as they are about in the same size range of the smallest enteropathogenic viruses.

Both, coliform bacteria as well as somatic coliphages were present in high concentrations in the river waters and therefore allowed a good calculation of the log removal for the particle removal treatment steps.

In the three water works, river water treatment consisted of a rapid sand filtration followed by a slow sand filtration of a few days.

Results for all three water works showed a log removal of 2.5 to 3.0 log for the indicators. This removal together with the pathogen concentrations in the river water did not allow to reach a health based target of 10-4 infections per person per year. Therefore, further treatment or disinfection steps or combinations of these are required to reach this goal. This is practice in Germany anyway, where the water after soil passage is not used as drinking water directly but is further treated in a multi-barrier process.

**66 Forecasting the Un-seeable : understanding the lifecycle of *Legionella pneumophila* in the premise plumbing system**

Alexis L. Mraz, Kerry Hamilton, Heather Murphy, Mark H. Weir

Temple University, The Ohio State University, Arizona State University

*Legionella pneumophila* (*L. pneumophila*) is the most common etiological agent of waterborne disease in the United States and is responsible for Legionaries' Disease, a severe, sometime fatal pneumonia. *L. pneumophila* thrives in biofilms, where it establishes an endosymbiotic (mutually beneficial) relationship with protozoa, in which *L. pneumophila* lives within the protozoa, providing the bacteria protection from disinfectants, such as residual chlorine. While there is a plethora of information regarding how *L. pneumophila* interacts with protozoa, this information has yet to be utilized to create a means of predicting its persistence and growth in water systems. Many genes and gene complexes have been identified in the lifecycle of *L. pneumophila*. However, few studies have determined how environmental stressors, such as residual disinfectants, affects gene expression. To our knowledge, no studies have modeled the change in *Legionella* bacteria concentration due to genetic changes.

We have developed a predictive model of *L. pneumophila*, accounting for variations in persistence and infectivity due to the genetic changes in the bacteria, which occurred as a result of exposure to residual chlorine in the premise plumbing system. This model provides initial guidance for more targeted treatment of *L. pneumophila* in water systems, but more importantly, provides a means of quantifying *L. pneumophila*'s growth and persistence in drinking water biofilms. This model examines the lifecycle of the bacteria under a variety of conditions including changes in piping material and hot water set points. This project informs how the built environment affects the proliferation and persistence of *L. pneumophila*, which can subsequently be used to model the effects of *L. pneumophila* on human health.

## SESSION 10 – ANTIMICROBIAL RESISTANCE

**67 Spread of Antimicrobial Resistance in Raw and Treated Sewage from Varied Sources in Brazil: a Metagenomic Approach for Hospital, Animal Husbandry and Domestic Wastewater Samples from São Paulo, Brazil**

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Spread of antimicrobial resistance is facilitated by horizontal gene transfer among bacteria. As most bacteria are not cultivable, this study aimed to assess the resistance scenery of sewage from several sources, searching for resistance and mobility-encoding genes using a culture-independent approach. In a one-year period, 500-mL sewage samples were collected monthly from (a) one Sewage Treatment Plant (STP), including raw sewage, treated effluent and reuse water; (b) one human hospital; (c) one veterinary hospital; and (d) one animal husbandry. Samples (n=121) were filtered through 45µm membranes and DNA was directly extracted using a commercial kit. PCR was performed for the

search of class 1 integrases (*intI1*) and genes encoding resistance for  $\beta$ -lactams (ESBL, AmpC and carbapenemases), Fluoroquinolones (PMQR, efflux pump OqxAB), Polymyxins (MCR), Aminoglycosides (RNA methylases) and Fosfomycins. From 32 genes searched, 26 were detected (11 in reuse water). All sampling sites revealed the presence of class 1 integrase, CTX-M (groups 1, 2 and 9), QnrB, QnrS, OqxAB and MCR-3. CTX-M-8, CMY, KPC, OXA-48, QnrD and MCR-1 were present at more than 75% of sampling sites. Some rare genes were also detected, like rmtD and RmtG at 65% of sites and *mcr-2* at the animal husbandry. The frequency of detection of each gene in each site was, in general, between 80% and 100%. Results show that raw and treated sewage, including reuse water, harbor resistance genes encoding for last-choice antimicrobials and class 1 integrons, which are frequently involved in horizontal gene transfer and spread of resistance. Moreover, several sceneries were implicated, including human and animal clinical settings, animal husbandry and the community. Thus, prevention of antimicrobial resistance spread should focus on the One Health concept, generating the awareness that all fields are responsible and need to establish effective policies to reduce this growing public health problem.

## **68 Antimicrobial Resistance and Virulence of *Enterococcus faecalis* and *Enterococcus faecium* Isolated from Wastewater Treatment Process**

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Enterococci are gram-positive bacteria, which form a part of the natural flora in the intestinal tract of human and animals, have been used as an indicator of fecal pollution of recreational waters. Some enterococci are known to be virulent and *Enterococcus faecalis* and *E. faecium* are most frequently associated with a range of enterococcal diseases in clinical settings. We monitored these important species of *Enterococcus* at a municipal wastewater treatment plant in Japan from January to June 2018 as a major source of pollution of water environment which has not yet been well investigated. In the monitoring, we focused on the changes of antimicrobial resistance and virulence of the target species in the standard activated sludge process. Among 581 enterococcal isolates from the treatment plant, 10.7% and 25.6% were identified as *E. faecalis* and *E. faecium*, respectively. The 211 isolates of these two species were examined for determination of MICs of twelve antibiotics used for medical treatment of enterococcal diseases, identifying only one isolate of *E. faecium* resistant to Vancomycin (i.e. VRE). On the other hand, the resistant rates of the isolates to ciprofloxacin (46.4%) and erythromycin (74.9%) were quite high with no significant changes in the treatment process, commonly to other antibiotics. The same pool of 211 *Enterococcus* isolates was further examined for detection of virulence genes (*gelE*, *asa1*, *esp*, *cylA* and *hyl*) using PCR analysis and 28.9% of the isolates possessed any of these genes with the highest prevalence (25.1%) for *gelE*. In addition, almost all (95.1%) of the isolates, from which at least one virulence gene was detected, showed resistant to one or more antibiotics. This result indicates that clinically important strains of enterococci, which are both virulent and resistant to antibiotics used for medical treatment, would be discharged from municipal wastewater treatment plants without proper disinfection.

## **69 Modeling Sunlight Degradation of Antibiotic Resistance Genes in the Environment**

Andrea I. Silverman, Fiona B. Dunn

New York University

The prevalence of antibiotic resistant bacteria (ARB) and the genetic materials that confer their resistance [i.e., antibiotic resistance genes (ARG)] is a growing global public health threat. One strategy to control the spread of antibiotic resistant pathogens is to prevent dissemination of ARB and ARG to the environment, for example through wastewater treatment. However, despite the ability of commonly used disinfectants to inactivate ARB, traditional treatment systems were not designed to

remove ARG from sewage, resulting in discharge of ARG to the environment. Therefore, the overarching goal of this research is to describe and model ARG decay in the environment with exposure to natural stressors, with a focus on photolysis by sunlight.

Sunlight is an important cause of virus and bacteria inactivation in sunlit waters, and sunlight-driven direct and indirect photolysis mechanisms are responsible for degradation of many organic molecules including nucleic acids. The focus of this study was to evaluate sunlight photolysis of the *vanA* gene, a section of plasmid-bound DNA that confers vancomycin resistance to gram-positive bacteria (e.g., *Enterococcus faecium*). Controlled, simulated sunlight experiments were conducted in the laboratory to determine the decay rates of intracellular and extracellular *vanA*. Photolysis rates were quantified by qPCR, and depended on the qPCR amplicon length, which has critical implications for the methodology used for surveillance of ARG in the environment. Decay curves from intracellular ARG photolysis experiments displayed a lag-phase before degradation, signaling the involvement of bacterial DNA photo-repair processes in delaying ARG decay. Sunlight photolysis rates of *vanA*, as quantified by qPCR, were orders of magnitude slower than those of bacteria and viruses. Lessons from *vanA* decay can be applied to other ARG. Continuing research is being conducted to determine how photolysis rates determined using qPCR reflect gene integrity and function.

## **70 Correlation of antibiotic resistance genes with crAssphage in an impacted urban watershed**

Elyse Stachler, Katherine Crank, Kyle Bibby

Eawag, University of Notre Dame

Antibiotic resistance is a growing problem that is often exacerbated by spread through environmental reservoirs. In order to help combat antibiotic resistance spread through the environment, accurate monitoring of ARG presence in natural waters is necessary. Previously, the human gut-associated bacteriophage, crAssphage, was shown to correlate with antibiotic resistance genes (ARGs) based on metagenomic observation. In this study, occurrence of crAssphage and ARGs was examined in an impacted urban watershed to understand the correlation that exists between them. For 30 days, daily water samples from a stream impacted by human fecal pollution (from combined sewer overflows during rain events) was collected and the concentrations of selected ARGs and crAssphage were measured by digital droplet PCR (ddPCR). ddPCR is an emerging technology that exhibits many benefits including detection of low copy number genes, decreased inhibition, and quantification independent of standard curve generation. The stream exhibited high concentrations of ARGs and crAssphage markers throughout the study that were statistically significantly higher during wet weather events. Correlations were also determined between ARGs and previously published qPCR data of human fecal indicators, including crAssphage. Quantification of crAssphage by ddPCR differed from qPCR values, resulting in lower correlation with ARGs when using crAssphage data generated with qPCR ( $r_{Spearman} = 0.40-0.53$ ) than with ddPCR ( $r_{Spearman} = 0.84-0.89$ ). This study emphasizes the importance of using compatible technology platforms when making quantitative comparisons and cautions against making correlations across platforms. This study also demonstrates that crAssphage presence can be an indicator of ARG presence in impacted environments, expanding the utility of crAssphage in water quality management.

## **71 Infectious phage particles packaging antibiotic resistance genes found in fish, shellfish and seawater**

Pedro Blanco-Picazo, Clara Gómez-Gómez, Daniel Toribio-Avedillo, Lorena Rodríguez-Rubio and Maite Muniesa

University of Barcelona

Bacteriophages are the most abundant and ubiquitous biological entities in the planet. These viruses, with lytic or lysogenic life cycles, can kidnap and package part of their host's genetic material, including antibiotic resistance genes (ARGs). After infecting a new host, phages can potentially transfer these genes by transduction, contributing to a rapid expansion of resistances among pathogenic bacteria. Phage particles containing ARGs have been identified in different environmental and agricultural settings, and in human biomes.

In this study, a variety of fresh fish and shellfish (from Atlantic Ocean and Mediterranean Sea), as well as frozen fish were analysed for the presence of eleven ARGs (*blaTEM*, *blaCTX-M-1*, *blaCTX-M-9*, *blaOXA-48*, *blaVIM*, *qnrA*, *qnrS*, *mecA*, *arma*, *sul1* and *tetW*) in the isolated phage DNA fraction. The study was completed with the analysis of seawater samples from different points of the Barcelona's coast.

ARGs were quantified by qPCR before and after propagation experiments, in an *Escherichia coli* strain, to evaluate the ability of the ARG-containing phages to infect and propagate in a bacterial host. Few samples showed ARGs in phage DNA by direct analysis and the number of positive samples increased after propagation, indicating that at least part of the isolated phage particles were infectious. The most abundant ARG in all the matrices was *tetW*, followed by β-lactamase genes. ARGs in phage DNA in seawater showed a low presence, although again *tetW* was the most prevalent.

This is the first study to reveal the presence of ARGs in phage DNA in marine environments, with particles accumulated in fish and shellfish suitable for consumption. Phage particles appear as a vehicle of transmission of ARGs from the sea environment through the food chain.

## **72 Antibiotic resistance genes in bacteriophages from rivers in a temperate climate**

Tristan M. Nolan, Liam Joseph Reynolds, Laura Sala-Comorera, Anjan Venkatesh, Wim G. Meijer  
UCD School of Biomolecular and Biomedical Science, University College Dublin, Ireland

Faecal pollution of surface water can result in surface waters being contaminated with clinically important antibiotic resistance genes (ARG). Antibiotics are the backbone of modern medicine, with an increasing prevalence of antibiotic resistance threatening to undermine this essential resource. The role of bacteriophages in the dissemination is becoming more evident, as bacteriophages can obtain and transfer ARG via transduction. Rivers that flow through agriculture and urban areas are subjected to different environmental pressures that can shape the structure of the microbial community, impacting on antibiotic resistance loading.

We aimed to evaluate the level and source of faecal pollution and to assess levels of clinically relevant ARGs in bacterial and bacteriophage fraction from rivers under different land use in a temperate climate.

Water samples were taken from two rivers and two streams influenced by different land uses (urban, agriculture and industry). Faecal indicator organisms including somatic coliphages were enumerated, as were faecal microbial source tracking markers (human or ruminant). Levels of genes conferring resistance to β-lactams, fluoroquinolone and sulfonamides were determined from bacteria and bacteriophage fractions.

Faecal indicator levels ranged over three orders of magnitude and the human marker was highest in urban streams. The human marker correlated with ARG levels in bacteria, but not in bacteriophage

fractions. Respectively, 100% and 84% of the bacterial and bacteriophage fractions were positive for the tested ARGs. In general, the bacteriophage fractions had lower ARG levels than the bacterial fractions by one or more orders of magnitude. No significant differences between rivers flowing through different land use areas were observed. Though bacteriophage fractions had lower ARG levels they may act as a reservoir for the transfer of ARGs to pathogens via transduction. These preliminary results offer an insight on the contribution of bacteriophages to ARG pollution of rivers and streams in a temperate climate.

### **73 Antimicrobial Resistance in the Environment: Perspectives from the Water Quality and Health Programme at the Water Research Commission (WRC), South Africa**

Eunice Ubomba-Jaswa and Nonhlaphela Kalebaila

Water Research Commission

For the last 5 years, The Water Research Commission through its water quality and health programme has funded intensive research on antimicrobial resistance (AMR) in the environment with specific emphasis on various types of water. Treated drinking water from treatment plants as well as household harvested rainwater have been found to be a medium for the development and dissemination of drug resistant pathogenic organisms and resistant genes. Surface waters such as estuaries as well as riverbed sediments have a high prevalence of multiple antibiotic resistant (MAR) bacteria of various types including *Escherichia coli* and *Vibrio spp.* Published data on AMR has occurred in 7 out of the 9 provinces in South Africa and reveals a clear link between environmental, clinical and farm settings through shared ARGs and ARGDs. Still to be established is a geospatial and temporal inventory of antimicrobials, metals, genes and biocides released into the environment which would be beneficial towards understanding the relative role that each might play in driving AMR in the environment. The ecological relevance of the different concentrations of AMR drivers in the environment (at sub-lethal concentrations and different matrices) is also critical in an understanding where resistance is likely to develop. Continuous AMR research is further spurred on by the need for a robust set of research evidence to facilitate the inclusion of an environmental perspective and action points into the South African National AMR strategy which currently has a focal point on the health/clinical perspective only like a number of AMR strategies worldwide. Secondly, as the one health approach gains momentum there is also a need for stakeholders involved in environmental issues to have a greater understanding of their contribution to the exacerbation of AMR and truly integrate with other disciplines to ensure research and data output leads to policy development and implementation that can have real and measurable impact.

# **POSTER ORAL PRESENTATIONS (POP)**

## **POP SESSION MONDAY**

### **POP 01 Exploring unidentifiable RNA virus sequences from metagenomic analysis of domestic wastewater**

Shinobu Kazama (1), Hitoha Moriyama (2), Yoshifumi Masago (3), Masahiro Otaki (2)

(1) The University of Tokyo, (2) Ochanomizu University, (3) National Institute for Environmental Studies

Viral metagenomic analysis using massive parallel sequencing generates huge number of unidentifiable sequence reads that does not hit any nucleotide sequences by BLASTn search (no-hit reads). Those no-hit reads may contain genome sequences of undiscovered human enteric viruses. To reduce sequence reads from bacteriophages and plant viruses that are abundant in domestic wastewater, we carried out metagenomic analysis targeting (+)ssRNA viruses with poly-A tail (selective analysis) for six domestic wastewater samples collected in Japan. Obtained no-hit reads were assembled into contigs, and concentration of virus genome with those sequences was quantified using real-time RT-qPCR assays. We also carried out metagenomic analysis targeting whole viruses (non-selective analysis) for the same samples. The mean ratio of (+)ssRNA virus reads to all reads was significantly increased by using the selective analysis (3.01 % vs 0.08%), which confirmed that (+)ssRNA virus genomes were successfully concentrated. Seventy-two contigs in total were assembled from the 2.5 million no-hit reads obtained by the selective analysis, and 5 out of the 72 contigs contained sequence reads from multiple samples (448 to 2,251 nucleotides). These contigs did not hit any sequences in the database by BLASTn search. Real-time RT-qPCR assays targeting 3 of the contigs (A, B, and C contigs) were developed and used to quantify viral genomes with the contig sequences from different 16 wastewater samples. As a result, the A and C contigs were detected in all samples with concentration of 101.9 -2.7 and 103.8-6.0 copies/mL, respectively. The B contig was detected in 50% of the samples with concentration of 101.8-4.0 copies/mL. These results suggested that novel viruses with the three contig sequences are present in Japanese wastewater at relatively high concentrations (comparable to Norovirus, Pepper mild mottle virus, or Laverivirus).

### **POP 02 Effects of different chlorine dioxide concentrations on microbial communities in drinking water**

Philipp Proksch, Christina Fiedler, David Kerschbaumer, Christoph Schönher, Marija Zunabovic-Pichler, Ernest Mayr, Reinhard Perfler

Institute of Sanitary Engineering and Water Pollution Control - University of Natural Resources and Life Sciences, Vienna

Various chlorine compounds are used for disinfection of drinking water and to inhibit bacterial regrowth, with chlorine dioxide being one of the most widespread. To investigate the effect of chlorine dioxide on drinking water microbial communities and its interaction with UV-irradiation in terms of biostability (i.e. increase in cell number), several combinations and concentrations of these disinfection methods were multiply prepared and incubated at 15°C for two weeks. Using High-Throughput-Sequencing (HTS) of the bacterial 16S rRNA gene and Flow Cytometry the microbial community structure and cell counts were assessed.

As expected, within samples processed immediately after UV-disinfection no shifts in the microbial community could be observed. After two weeks of incubation microbial composition changed clearly, with a strong dependence on the disinfection method used. Following untreated water, low chlorine dosage (0,05 mg/l) and UV-only showed the lowest biostability, but with strong community shifts in the UV-only samples. In samples with high chlorine dioxide concentrations (0,15 mg/l), the relative community structure was only slightly altered compared to the initial samples. The lack of viable cells indicates cell debris to be remaining, thus showing the importance of estimated absolute abundance values and intact cell counts in community analysis of disinfected samples.

Although biostability was slightly better than without UV-treatment, most substantial changes were observed in UV-treated samples with low chlorine addition. The community shifted towards low diversity and a domination of members of Comamonadaceae (now in Burkholderiaceae) and Methylphilaceae.

These results indicate, that unless chlorine dioxide concentrations are high enough to entirely suppress regrowth, chlorine dioxide additions to UV disinfected water may impair biostability in terms of cell numbers and diversity. Therefore, considerations on the interdependency of these disinfection methods should always be taken into account before they are implemented in waterworks.

### **POP 03 From hospital wastewater to receiving water bodies: a comparative shotgun metagenomics of aquatic environmental resistomes**

Ekwanzala, Mutshiene Deogratias (1); Dewar, John Barr (2); Kamika, Ilunga (2); Momba, Maggy Ndombo Benteke (1)

(1) Tshwane University of Technology, (2) University of South Africa

It has been reported that aquatic environments are receiving high loads of untreated hospital and municipal wastewater that can be important hotspots for the dissemination of antibiotic resistance bacteria (ARB) and genes (ARGs) into the environment making surface water unfit for drinking or irrigation water. Therefore, the aim of this study was to compare and characterise the trail of ARB, ARGs and other ARGDs (plasmids, transposons, bacteriophages) from hospital and municipal wastewater to waterbodies using comparative shotgun metagenomics in order to uncover their distribution and dissemination. Shotgun metagenomics was used to determine the distribution of ARGs in hospital effluents, wastewater influents, activated sludge, wastewater effluents, surface water bodies and benthic sediments. We subsequently interrogated the generated data from HiSeq X Ten platform using bioinformatic analysis to reveal ARGs abundances, identification of present taxa, presence of pathogenic bacteria as well as the mobile genetic elements. We also explored co-occurrence using network analysis. We found that there was no significant difference in abundance and composition between influent of wastewater and sediments. Higher ARGs loads were found in influents of wastewater followed by riverbed sediments while activated sludge resistomes were more diverse. Several clinically important ARGs such as *mcr-1* and *optrA* were detected and their abundance differed between host species and matrix. Gram-negative bacteria harboured most ARGs (71%), with fewer genes from Gram-positive Actinobacteria and Bacilli (Firmicutes) (9%) and were impacted by the taxonomic composition of the wastewater, while solid samples showed more diverse resistomes. Effluents of wastewater and sewage sludge also contained a substantial load of ARGs, which may lead to the enrichment of the resistomes in rivers and agricultural soils, respectively. We have also found that ARGs co-occurred between all matrix analysed using network analysis.

### **POP 04 Virome and resistome based on metagenomic analyses of influent and effluent waters from wastewater treatment plants**

Alba Pérez-Cataluña (1), Enric Cuevas (1), Walter Randazzo (1, 2), Gloria Sánchez (1)

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Waterborne viruses are a significant cause of human disease and their detection is heavily dependent on PCR-based methods that require reference sequences for primer design. In this pilot study, a total of eight samples were collected upstream and downstream of four wastewater treatment plants (WWTPs). Viral particles were enriched from water concentrates using a filtration and nuclease digestion procedure prior to DNA/RNA extraction and preparation of sequencing libraries for each individual water sample. Initial bioinformatic analyses were performed by CosmosID software. Total read averages of 0.41% and 0.11% were assigned to viruses in the influent and effluent water samples, respectively. On average, 29.3%, 1.3%, and 1.1% of reads in the influent water samples and 1.5%,

0.6%, and 0.7% in the effluent samples were assigned to bacteria, protozoa, and fungi, respectively. Additionally, cleaned reads were annotated using an in-house database. This annotation showed an increased percentage of viral reads, with 0.74% and 0.3% on average in the influent and effluent water samples, respectively. As a richness indicator, the number of viral families was determined to be 19 in the influent and 17 in the effluent water samples. Additionally, non-viral reads were processed using the ResFinder and CARD databases to identify genes associated with antimicrobial resistance (AMR). Interestingly, the most abundant AMR genes identified by both databases corresponded to resistance for macrolides, chloramphenicol, and aminoglycosides. The proposed metagenomic procedure constitutes a feasible approach for virome and resistome surveillance in influent and effluent water samples.

## **POP 05      The power of short-amplicon high throughput sequencing for water quality monitoring: understanding risk and its sources**

Rebekah Henry, David McCarthy

Environmental and Public Health Microbiology Laboratory (EPHM Lab), Monash University, Australia

Monitoring faecal contamination levels in recreational waterways has traditionally relied on indicator organisms. The use of such indicators was reinforced by high quality epidemiological studies that found links between indicator concentrations and adverse human health impacts. However, recent studies have demonstrated that simple indicators do not necessarily correlate with human health risks in recreational waterways when multiple sources of faecal contamination exist. This has led to the exploration of other indicators that might be more suitable.

We propose that to create robust links between water quality and human health outcomes, we must rely on multiple lines of evidence, especially for systems that receive a multitude of faecal sources. Our approach is to use amplicon sequencing to create bacterial, protozoan and viral community profiles that can be used (1) estimate risk to users of recreational waterways and (2) determine the sources of faecal contamination in the waterbody.

Over the past 8 years, we have collected 530 samples from a variety of surface waters and analysed them for indicator organisms as well as bacterial, protozoan and viral reference pathogens. Concurrently, high throughput amplicon sequencing of the 16s gene was conducted. SourceTracker analyses was conducted to compare the water community profiles to those of regionally specific animal and human faecal source samples.

We observe highly significant correlations between *E. coli* and enterococci (as measured by IDEXX) and the proportion of the 16s community that belong to *Enterobacteriaceae* and *Enterococcaceae*. The proportion of each community that were faecal-associated were also statistically correlated with our IDEXX *E. coli* and enterococci datasets. Furthermore, our results demonstrated significant links between 16s community profiles and the measured reference pathogen concentrations (full details in the presentation), demonstrating the potential of such methods to be used for measuring and mitigating risks in recreational waterways.

## **POP 06      An exploration of the disease burden due to *Cryptosporidium* in consumed surface water for sub-Saharan Africa**

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*Cryptosporidium* is a leading cause of diarrhoeal disease. Its burden, however, remains uncertain, especially for the low-income regions that are most severely affected. Quantifying the role of specific exposure pathways is important for effective disease mitigation, but limited data availability has hampered such assessments. New data produced by the Global Waterborne Pathogen model (GloWPa)

allowed us, for the first time, to explore the contribution of surface water consumption to the overall burden caused by *Cryptosporidium*. We conducted a quantitative microbial risk assessment (QMRA) for sub-Saharan Africa using GloWPa-produced data on oocyst surface water concentrations. In the absence of large-scale exposure data, we estimated the number of people consuming surface water in this region, and included both direct consumption and consumption of surface water from a treated piped supply. Our results provide national level estimates of the disease burden (in disability adjusted life years, DALYs) caused by *Cryptosporidium* in consumed surface water. For sub-Saharan Africa overall this exposure pathway could be responsible for  $4.3 \times 10^7$  (95% uncertainty interval [UI]  $7.4 \times 10^6 - 5.4 \times 10^7$ ) cases representing  $1.6 \times 10^6$  (95% UI  $3.2 \times 10^5 - 2.3 \times 10^6$ ) DALYs. Relative disease burden (DALYs per 100,000 persons) varies widely, ranging between 1.3 (95% UI 0.1–5.7) for Senegal and  $1.0 \times 10^3$  (95% UI  $4.2 \times 10^2 - 1.4 \times 10^3$ ) for Eswatini. The findings indicate that the vast majority of cases occur in the population consuming surface water directly, while the immunocompromised population carries the largest share of the burden. Our estimates represent approximately 19.4% of the overall cryptosporidiosis burden, and 59% of waterborne cryptosporidiosis cases in sub-Saharan Africa, which appears to be reasonable when compared to the available literature. This study provides quantitative insight into the role of surface water as a transmission route for *Cryptosporidium* in low-income settings, and demonstrates the potential value of modelled pathogen concentration data for epidemiological research.

**POP 07     Genotype analyses of *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Aspergillus fumigatus* isolates from drinking water reveal similar genotypes with patient strains**

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It has been observed that the opportunistic pathogens *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Aspergillus fumigatus* are infrequently present in drinking water in the Netherlands. However, this finding does not automatically mean that the drinking water strains of these organisms are pathogenic, as environmental strains can be genetically different to clinical isolates and lack the ability to infect humans. Therefore, a study was conducted in which drinking water strains of *P. aeruginosa*, *S. maltophilia* and *A. fumigatus* were isolated from drinking water samples taken from the distribution system of nine different treatment plants in the Netherlands. The isolated strains were genetically characterised, using multilocus sequence based typing, whole genome sequencing or microsatellite analysis. The obtained genotypes were compared with genotypes of patient strains that were in Dutch and/or international databases. In total, 33 strains of *P. aeruginosa*, 107 strains of *A. fumigatus* and 1681 strains of *S. maltophilia* were isolated from distributed drinking water. The 33 strains of *P. aeruginosa* belonged to eleven different sequence types, from which seven sequence types had also been isolated from patients. 50 of the 1681 strains of *S. maltophilia* were genotyped, but none of these corresponded to genotypes from patients. Six of the 107 *A. fumigatus* strains were azole-resistant, which poses an increased risk for public health. One of these six strains had a genotype that was similar to a clinical isolate. In addition, the genotype of an azole-sensitive strain was also similar to a clinical *A. fumigatus* isolate. Overall, we conclude from our study that the presence of *P. aeruginosa* and *A. fumigatus* in distributed drinking water in the Netherlands can pose a threat to public health. Still, additional research is required to weigh the risk of drinking water transmission compared to transmission from other sources for these two pathogens.

## **POP 08 Standardization and detection by the technique of real-time PCR of *H. pylori* in drinking and raw surface water**

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Empresa de Acueducto y Alcantarillado de Bogotá EAAB ESP

Helicobacter pylori infects the stomach of man and has been associated as a causative agent of gastritis, peptic ulcer and gastric cancer. The fecal-oral route of transmission with water contaminated with faeces is the main source of infection in countries with low socioeconomic levels. Recent studies report a prevalence of Colombia for *H. pylori* infection of 83.1%, which affects gastric cancer prevalence of 17.2%. in Colombia, this cancer represents the first cause of death from this disease in men and the third in the women. In Colombia there is no standardized technique to identify Helicobacter pylori in water, so this work aims to standardize the concentration, extraction and real-time qPCR with different oligonucleotides: 16S rRNA, GLMM, ureA, cagA and vacA, for identifying the presence of Helicobacter pylori water for human consumption. Three oligonucleotides were standardized: 16S rRNA, ureA y vacA. These genes are related to the presence of *H. pylori* in gastric cancer. Centrifugation was the best method of concentration of the microorganism. The attributes of the real-time detection were calculated for each oligonucleotide with which concentrations of 10 copies / reaction of *H. pylori* in water can be detected. 100 samples of drinking water and 100 samples of untreated surface water were made. The presence of Helicobacter pylori was detected in drinking water and in raw water. The objective of this study is to standardize the technique to look for alternatives for disinfection in drinking water such as UV light, Ozone or other disinfectants such as Titanium Dioxide, likewise for the taking of actions for the care of water sources for treatment.

## **POP 09 Faecal indicator removal mechanisms in aerobic granular sludge systems**

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Aerobic Granular sludge (AGS) is an emerging treatment technology aimed to efficiently remove organic matter and nutrients. A previous study showed that a full-scale AGS system achieved similar removal efficiencies of faecal indicator microorganisms (FIM) as the conventional activated sludge (CAS) system, namely 1 to 2 Log10 removal of F-RNA specific bacteriophages, *E. coli*, *Enterococci*, and thermotolerant coliforms. However, it is unknown what the main pathogen removal mechanisms of the AGS systems are, and in how far they are different from the known CAS system removal mechanisms such as protozoa predation, enmeshment in the sludge or cell lysis. This study aimed to elucidate the removal mechanisms by comparing the fate of *E. coli* in the different phases of an AGS laboratory-scale sequencing batch reactor. The AGS reactor was operated with an anaerobic feeding phase followed by a more extended aeration phase. Effluent was withdrawn after a short settling phase. Synthetic wastewater spiked with a known concentration of *E. coli* ATCC25922 ( $10^7$  CFU/100mL) was used as influent. Samples were taken from the influent, effluent, and at the end of the anaerobic and aerobic phases, and of the sludge. Bacteria were quantified using the membrane filtration method. Protozoa growth and predation were determined by optical microscope observation (4x) and fluorescence microscopy using pre-stained *E. coli* (dsGreen). The system achieved a total removal of 2.5 Log10 *E. coli* on average. During the anaerobic phase no removal was observed, after the aeration phase a 1.0 Log10 reduction was measured. Settling contributed to the remaining removal, caused by attachment of the *E. coli* to the granules. Image analysis of the granules showed the vacuoles of stalked ciliates full of pre-stained *E. coli* and also showed some accumulation on the granule surface. Further studies need to be done to determine the effect of cell lysis by analysing another type of FIM (i.e., MS2).

## **POP 10 Shorebirds, a possible source of *Campylobacter* spp. in shellfish?**

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The microbiological quality of coastal environments can be affected by fecal pollution from urban and agricultural sources and by wildlife. The aims of this study were first to evaluate the occurrence of *Campylobacter* spp. in a shellfish-harvesting area and its catchment, and their occurrence in shorebird feces, and secondly, to compare isolates from shellfish, river, and seabird fecal samples.

The shellfish-harvesting area is located in the Brittany region in France, characterized by intensive livestock farming (cattle, pig, and poultry). Shellfish (n=120), marine water (n=12), river water (n=96), and sediment (n=14) samples were collected from February 2013 to January 2015. Fresh droppings from 12 wild bird species (n=457) were collected from December 2016 to December 2018 at this site and three additional coastal sites in Brittany. *Campylobacter* spp. was detected using ISO-10272:2006 method and species identified by MALDI-TOF. The genomes of a selection of isolates were sequenced by Illumina MiSeq. Spades(v.3.12.0) was used for assembly and SeqSphere (Ridom) for ST recovery.

*Campylobacter* spp. was isolated in 39.2% of shellfish batches, 58.3% of marine water, 84.4% of river water, 28.6% of sediment samples and 51.6% of bird droppings. More precisely, *Campylobacter lari* was predominantly isolated in shellfish batches, marine waters and bird droppings (38.3%, 50%, and 26.5% of samples analysed, respectively) whereas *C. jejuni* and *C. coli* were more frequently isolated in river waters (39.6% and 50% of samples analysed, respectively). Analysis of genomes of *C. lari* isolates shows a high genetic diversity and the identification of new STs. Only part of the STs from shellfish were close to STs isolated from the upstream catchment.

This study highlights the high prevalence and dominance of *C. lari* in shellfish and shorebird feces. Within the next steps, the WGS data will undergo further analyses, by considering e.g. cgMLST and the detection of virulence genes.

## **POP 11 Field verification of the bag-mediated filtration system version 2 in Kenya and Pakistan**

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University of Washington, Kenya Medical Research Institute, National Institute of Health Islamabad, National Emergency Operations Center, World Health Organization, Bill & Melinda Gates Foundation, PATH

Environmental surveillance (ES) plays an important role in global poliovirus (PV) eradication. The bag-mediated filtration system (BMFS) yields enhanced PV ES sensitivity when compared to the gold-standard World Health Organization aqueous polymer two-phase separation method (two-phase). Field-validated BMFS version 1 (v1) was recently modified to version 2 (v2) to improve PV recovery, ease-of-use, and cost. This study verified BMFS v2 for PV ES by comparing PV detection in v1, v2, and two-phase samples collected in Kenya (Mar-Jul 2018; n=18) and Pakistan (Jan-Mar 2019; n=21).

Samples were assayed by the WHO algorithm for Sabin-like (SL) PV, wild PV (WPV), and vaccine-derived PV (VDPV) types 1, 2, and 3. In the Kenya verification study, v1 and v2 resulted in statistically more frequent detection of SL1 ( $p=0.125$  and 0.008, respectively) and SL3 ( $p=0.012$  and 0.003, respectively) when compared to two-phase. v2 resulted in more frequent SL1 and SL3 detection compared to v1 ( $p=0.063$  and 0.375, respectively). There was no statistical difference in SL2 detection between sample types. On 21-March, VDPV2 was detected in v1 and two-phase samples; v2 results were inconclusive. No WPV was detected. In the ongoing Pakistan verification study, results are available from 13 sampling events. v1 and v2 resulted in similar or more frequent detection of SL1 ( $p=0.688$  and 0.219, respectively), SL3 ( $p=1$  and 0.125, respectively), and WPV1 ( $p=0.625$  and 1, respectively) when compared to two-phase. No SL2 or VDPV were detected. Field personnel noted that the v2 sample collection, filtration, and laboratory processing is simpler and requires less time than v1. BMFS cost of goods decreased, though the price is not finalized as commercialization is ongoing. These field studies demonstrated that v2 results in comparable or in some instances improved PV detection compared to v1 and two-phase methods. Future BMFS surveillance activities should utilize v2 in lieu of v1.

## **POP 12    Rapid methods for HEV detection in environmental waters**

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Hepatitis E virus (HEV) represents an emerging infectious disease in the European Union, and the incidence of confirmed cases has been steadily increasing over the last decade. HEV is present in environmental waters worldwide, especially the zoonotic genotype 3 in industrialized countries. To detect HEV that may be present on different types of waters, we evaluate different procedures to concentrate HEV in untreated and treated sewage, bottled and tap water samples. To this end, bottled water samples were concentrated by filtration via positively charged nylon membranes as recommended by ISO 15216-1:2017. An aluminum hydroxide adsorption-precipitation procedure was used to concentrate HEV from influent and effluent water samples from a wastewater treatment plant (WWTP). Tap water samples were processed with a primary concentration via Dead End Hollow Fiber Ultrafiltration (DEUF), followed by a secondary concentration carried out through either polyethyleneglicol (PEG) precipitation or an ultrafiltration procedure. Moreover, two RNA extraction procedures, differing for binding nucleic acids to silica membranes (MN) or magnetic beads (NS), and different RT-qPCRs assays were compared in this work. The evaluated procedures resulted in average HEV recoveries of 9.4%, 15.2%, 19.9% and 16.9% in bottled, influent, effluent and tap waters, respectively, with a detection limit ranging from 103 to 104 IU/L. Finally, one-year pilot study was performed to monitor the prevalence of potentially infectious HEV in influent and effluent waters samples collected upstream and downstream of four WWTPs using a viability RT-qPCR assay based on platinum chloride pre-treatment.

## **POP 13    Rapid detection of *Legionella pneumophila* as screening tool for an improved outbreak management**

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Legionella are Gram-negative bacteria that can cause life-threatening pneumonia in humans called Legionnaires' disease. They multiply in warm water and can be dispersed into the air via cooling towers, evaporative recooling systems and hot water systems if bioaerosols are generated. In case of an outbreak, finding the exact source as quickly as possible is essential to prevent further infections.

To date, a detailed analysis of water samples by cultivation takes 7 – 10 days with a high rate of false negative results. Therefore, a German BMBF-funded project LegioTyper has been launched to establish rapid screening methods based on flow-based chemiluminescence microarray immunoassays for serotyping of *Legionella pneumophila* and molecular biological methods for the quantification of *Legionella* spp. by viability qPCR or alternatively heterogeneous asymmetric recombinase polymerase amplification. The Legiotyper instrumentation is an automated microarray analysis platform which is able to detect and serotype all *L. pneumophila* Sg1 subgroups directly in water or urine samples within 34 min by using a panel of 10 monoclonal capturing antibodies on a flow-based immunochip. Concentrations of all capture antibodies were adjusted to maximum specific signal and least cross-reactivity. Different sample types like urine and process water were analyzed successfully with the established panel. The detection limit for identification of *L. pneumophila* (e.g. Serogroup 1 subtype Bellingham) was  $2.2 \times 10^4$  CFU/mL in tap water. A concentration from 10-L water samples to 1 ml by monolithic adsorption filtration and centrifugal ultrafiltration was developed to improve the detection limit to 4 log units. Viability tests by molecular biological methods were performed to detect viable *Legionella* spp. and *L. pneumophila* in water samples. The established screening methods were implemented in a VDI guideline for a catalogue of measures in case of suspicion of emission-related legionellosis outbreaks.

#### **POP 14 Assessing the Transition Effects in a Drinking Water Distribution System Caused by Changing Supply Water Quality: An Indirect Approach by Characterizing Suspended Solids**

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To meet the increasingly stricter drinking water standards, the treatment processes have been widely upgraded to improve the supply water quality. However, the potential impacts of improved drinking water on the stabilized ecological niches within the drinking water distribution systems (DWDSs) and the associated water quality deterioration risks (e.g. Flint Water Crisis) have long been neglected. In the present study, such transition effects caused by changing the supply water quality that resulted from destabilization of biofilm and loose deposits in DWDS were investigated by analyzing the physiochemical and microbiological characteristics of suspended particles before (T0), during (T3-weeks) and after upgrading the treatments (T6-months) in an unchlorinated DWDS in the Netherlands. Our results demonstrated that after 6 months' time the upgraded treatments significantly improved the water quality. Remarkably, water quality deterioration was observed at the initial stage when the quality-improved treated water distributed into the network at T3-weeks, observed as a spike of total suspended solids (TSS, 50-260%), active biomass (ATP, 95-230%) and inorganic elements (e.g. Mn, 130-250%). Furthermore, pyrosequencing results revealed sharp differences in microbial community composition and structure for the bacteria associated with suspended particles between T0 and T3-weeks, which re-stabilized after 6 months at T6-months. The successful capture of transition effects was especially confirmed by the domination of *Nitrospira* spp. and *Polaromonas* spp. in the distribution system at T3-weeks, which were detected at rather low relative abundance at treatment plant. The methodology and knowledge presented in this study will be valuable for the water utilities worldwide for assessing potential transition effects and ensuring the quality and safety of drinking water supply.

## **POP 15 Fresh-cut wastewater disinfection by solar processes with iron-chelate (Fe3+-EDDHA)**

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The fresh-cut industry has recently emerged to serve the increased demand of population for ready-to-eat fresh food. Nevertheless, this activity is one of the major water consumers of the agro-food industry (>40 m<sup>3</sup>/ton of raw product). In this context, the application of an appropriate water reuse approach to reduce the water footprint (from vegetable cultivation to their processing) is necessary. Solar water disinfection treatments including solar photochemical (H<sub>2</sub>O<sub>2</sub>/solar) and photocatalytic (photo-Fenton) processes have shown to be an environmentally friendly process with high disinfection capability. Nevertheless, the efficiency of photo-Fenton at neutral pH is strongly reduced due to the iron precipitation, and research on new sources of iron including complexation with organic compounds to increase its disinfection capability has lately arisen.

The goal of this study was to investigate the use a commercial iron fertilizer (Fe<sup>3+</sup>-EDDHA) as source of iron for synthetic wastewater from fresh-cut industry (SFCWW) disinfection. This compound (EDDHA - ethylenediamine-N,N'-bis(2-hydroxyphenylacetic acid) is widely employed in agriculture to remediate iron chlorosis in crops. The assessment of the disinfection capability of the new iron-chelate alone (Fe<sup>3+</sup>-EDDHA/Solar) and in combination with H<sub>2</sub>O<sub>2</sub> (Fe<sup>3+</sup>-EDDHA/H<sub>2</sub>O<sub>2</sub>/Solar) has been investigated at laboratory scale (200 mL) under natural solar radiation in comparison with solar only and H<sub>2</sub>O<sub>2</sub>/solar. Two well-known waterborne pathogens in this industry as responsible of many outbreaks associated to the consumption of contaminated fresh-food have been used as model of microorganisms, *E. coli* O157:H7 and *Salmonella enteritidis*. Results showed the improvement of the solar disinfection efficiency by using very low amount of iron chelate (>2.5 mg/L of Fe<sup>3+</sup>-EDDHA), reducing > 5-Log of bacterial concentration in 30 min of treatment time. These results highlight the possibility of SFCWW reuse for irrigation with the simultaneous advantages of reduced the iron chlorosis, the water scarcity and operating costs.

## **POP 16 Contamination of cultured oysters with wild-type Rotavirus A and its relationship with gastroenteritis epidemic**

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Norovirus, a leading causative agent for acute gastroenteritis in the world, has been proved to circulate in human population, water environment, and oysters. Rotavirus is also the leading viral agent for pediatric gastroenteritis but its circulation in the environment remains unknown. This study aimed to reveal the temporal variation of Rotavirus A (RVA) level in cultured oysters and its relationship with gastroenteritis epidemic. Nine individual oysters were collected weekly at a bay in Miyagi, Japan, from September 2014 to July 2016 and total number of RVA in the oyster samples was determined by qPCR using universal primers/probes. The oyster samples were further examined for quantitative detection of two vaccine strains, Rotarix and RotaTeq. The number of wild-type RVA was calculated by subtracting the quantitative data of RVA vaccines from that of total RVA. Total RVA in the oysters had a similar seasonality to Norovirus, with higher concentrations in winter. RotaTeq strains were also found frequently in winter, although their concentrations were stable in sewage as a primary source of oyster contamination. Rotarix strain was hardly detected both in the oyster and sewage samples. The concentrations of RotaTeq strains, ranging in 1.9 ~ 3.4 log copies/g-Digestive Tissue, were so low (<1%) compared with total RVA. The concentration of wild-type RVA in the oyster samples was

neither significantly correlated to that in sewage nor to reported cases of rotavirus gastroenteritis around the studied bay. This is not consistent with the previous studies for norovirus, which showed significant correlations as a proof of its circulation. RVA is known to include both human- and animal-source strains; only human-source strains can be detected in sewage, while oysters could have been contaminated by both. No clear relationship between wild-type RVA in oysters and gastroenteritis epidemic is probably attributed to the existence of such animal-source strains.

**POP 17 Impact of Social Disparities on Microbiological Quality of Drinking Water Supply and Health of HIV/AIDS Infected Individuals: A Case Study in Ugu District Municipality of Kwazulu-Natal Province, SA**

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The present study investigated the impact of social disparities on the microbiological quality of drinking water and health of HIV/AIDS infected individuals. Two hundreds twenty (220) rural and 108 urban households of Ugu District Municipality were randomly selected during the period of the survey (between January 2008 and November 2009, and during April 2015). The results of the survey revealed the persistence of great disparity in the water supply as the majority of the rural dwellers indicated that standpipes located outside their homes (90.9%) remain their sole mode of access to drinking water, while in the urban area all households had pipes/taps inside their houses (100%). In terms of sanitation, 100% of urban dwellers were found to have proper sanitation facilities (flush toilets), while 98.2% of the rural dwellers were still using pit latrines, and 1.8% practiced open defecation. The episodes of diarrhoea were found to be high in rural areas (34.1%), while no episodes of diarrhoea were reported in urban areas. Significant correlations were shown between the incidence of potentially pathogenic bacteria isolated from chlorinated household-stored water and in stool specimens of HIV-positive patients with diarrhoea ( $r = P < 0.05$ ), using culture-based methods. A combination of molecular techniques was applied to isolates obtained from stored-water samples and stool specimens of HIV/AIDS-positive and non-HIV/AIDS negative patients with diarrhoea. Results revealed similar sequences of *Klebsiella* spp., *Klebsiella pneumoniae*, *Escherichia coli*, *E. coli* O55:H7, *Proteus mirabilis*, and *Shigella boydii*. With the exception of *Proteus mirabilis*, none of these pathogens were identified in stool specimens of non-HIV-infected individuals with diarrhoea. This study suggests a potential association between poor household drinking-water quality and diarrhoeic conditions of HIV/AIDS individuals living in the rural areas of the Ugu District Municipality of the KwaZulu-Natal Province.

**POP 18 Chemical and Microbiological Drinking Water Risks for Infants in Coastal Peru**

Miranda J. Delahoy (1), Sydney Hubbard (1), Mia Mattioli (1), Jackie Knee (2), Forest Altherr (1), Rebecca Hodge (1), María del Rosario Jaramillo Ramírez (3), Alisson Zevallos-Concha (4), Priya E. D'Souza (1), Parinya Panuwet (1), Carlos Culquichicón (4), Lilia Cabrera (5), Dana Boyd Barr (1), P. Barry Ryan (1), Andres G. Lescano (4), Joe Brown (2), Robert H. Gilman (6), Karen Levy (1)

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Unsafe drinking water poses numerous chemical and microbiological threats to child health. We explored drinking water exposures in 96 households with infants in Piura, Peru in 2016.

For each household we administered an exposure and health questionnaire, quantified arsenic in a 15ml drinking water sample using inductively-coupled plasma mass spectrometry, and quantified pesticides in 1L of drinking water using gas chromatography-tandem mass spectrometry. We collected 323 100ml drinking water samples from 96 households and assayed for *E. coli* using the IDEXX

Quantitray method. Stool samples were obtained from 94/96 infants and assayed for enteropathogens using the Luminex Multiplex Gastrointestinal Pathogen Panel.

We found high rates of microbiological and chemical contamination in household drinking water; most samples were piped drinking water. Of 323 water samples, 37% were positive for *E. coli*. Stored drinking water samples (69% of samples) had significantly higher odds of being positive for *E. coli*, (OR: 4.70, 95% CI: 2.15-10.30) and households with animals tended to have higher odds of *E. coli* in water (OR 1.97, 95% CI: 0.92-4.21); having a piped sewerage connection was protective against *E. coli* contamination (OR: 0.42, 95% CI: 0.20-0.89).

Arsenic was detected in 50% of 96 water samples; 25% of all samples exceeded the World Health Organization arsenic limit of 10 µg/l. Pesticides were detected in 58% of samples with atrazine being most commonly detected. Mothers reported diarrhea in 14% of infants; at least one enteropathogen was detected in stool in 68% of infants. We found significantly elevated odds of diarrhea in households with a water sample positive for *E. coli* ( $p<0.01$ ) and marginally higher odds of diarrhea in households with water samples with >10 µg/l of arsenic (OR: 3.00; 95% CI: 0.89-10.06). Drinking water contamination may pose a risk of acute and/or long-term adverse health outcomes in infants in Piura, Peru.

## POP SESSION TUESDAY

### POP 19 A novel method for modelling the risk of antimicrobial resistance

Shin Giek Goh, Peng Jiang, Charmaine Ng, Hongjie Chen, Laurence Haller, Francis Rathinam Charles, Huiting Chen, Xiao Liu, Karina Yew-Hoong Gin

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The overuse and misuse of antibiotics has led to the current global antibiotic resistance crisis. The concern of antimicrobial resistance (AMR) was previously focused at healthcare facilities. In recent years, the environment is gaining recognition for its role in spreading AMR. AMR can spread to the environment through several routes such as wastewater effluent discharges, animal manure application as fertilizer for crops, agricultural runoff, aquaculture, veterinary sources, etc. Challenges exist in quantifying the risk associated with AMR. One of the main problems is lack of dose response models available for pathogens which are resistant to antibiotics. In addition, very little is known regarding the health risks posed by antibiotic resistant genes (ARG). In this study, we propose a procedure to evaluate the relative risk of AMR based on antibiotic-resistant bacteria (ARB) and ARG. The evaluation of AMR risk involved data collection from expert surveys, data extraction from database (ARG Ranker; [https://github.com/caozhichongchong/arg\\_ranker](https://github.com/caozhichongchong/arg_ranker)), as well as reference to WHO's priority list for antibiotic resistant bacteria. A total of 40 surface water samples from various locations in Singapore with different land use were collected. The concentration of antibiotic resistant pathogens (i.e.. *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli* and *Enterococcus spp.*) resistant to 6 types of antibiotics were measured, together with 14 types of ARG. Regression models were developed to predict the concentration of ARB and ARG based on the environmental parameters. The risk of AMR for each sample was calculated based on the concentration and risk weightage assigned for each ARB and ARG. The AMR risk model developed in this study will be able to identify AMR hotspots and thus, provide better control and management of resistance emergence in the environment.

**POP 20 Modelling climate change impacts on microbial risks for a safe and sustainable drinking water system**

Ekaterina Sokolova (1), Sandra Lindqvist (2), Erwin Diener (1), Stephan Köhler (2), Mia Bondelind (1)

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The public impact due to waterborne illness can be extensive and cause severe economic consequences for society. There is a need for greater preparedness to deal with disruptions due to extreme weather and other effects of climate change that can affect the drinking water system. The aim of this work is to characterise the microbial risks in drinking water systems in order to facilitate sustainable management of water systems under conditions of changing climate. The climate change effects were studied on the example of two drinking water sources in Sweden – Lake Rådasjön in Gothenburg and Lake Ekoln in Uppsala. The future conditions (middle and end of the century) in these drinking water sources were simulated using three-dimensional hydrodynamic modelling; the outputs from the global circulation models regarding meteorological conditions were used as input data. The effects of increased air temperature on the seasonal density-driven circulation in the water sources were investigated. The changes in timing and duration of mixed and stratified conditions in these dimictic lakes were modelled. Understanding seasonal circulation in lakes is important in the context of drinking water supply, as water intakes are often located below the thermocline, and water density gradients impact the spread of pollutants. The effects of extreme precipitation events and consequential wastewater discharges on the microbial water quality in the water sources were also modelled. This work is performed within an on-going research project ClimAQua. In the context of drinking water management, this project contributes to correct identification of risks and prioritisation of mitigation measures, in order to facilitate well informed and transparent decision-making and efficient use of available resources. This research contributes to achieving the UN Sustainable development goals, e.g. Goal 6 Clean water and sanitation and Goal 13 Climate action

**POP 21 Two-year monitoring of norovirus and rotavirus present in suspended and dissolved forms in drinking water sources in Japan**

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This study aimed to investigate the present forms of norovirus GII and rotavirus A in surface water used for drinking water production. River water samples collected at a drinking water treatment plant (DWTP) monthly for two years, from 2017 to 2019, were fractionated by filtration through 10- and 0.45- $\mu\text{m}$ -pore-size membranes, and viruses present in suspended and dissolved forms were quantitatively detected by using a real-time PCR method. Norovirus GII was present in > 10- $\mu\text{m}$ - and 0.45–10- $\mu\text{m}$ -suspended and dissolved forms with detection rates of 32%, 59%, and 86%, respectively. Rotavirus A was detected more frequently than norovirus GII in each form (> 10  $\mu\text{m}$  suspended, 82%; 0.45–10  $\mu\text{m}$  suspended, 95%; dissolved, 100%). We also analyzed surface water samples from 21 DWTPs all over Japan twice a year (i.e. non-epidemic and epidemic seasons of gastroenteritis). Norovirus GII was detected in 36% and 71% of samples with the concentrations of up to 4.1 and 5.3 log<sub>10</sub> copies/L in dissolved form in non-epidemic and epidemic seasons, respectively, and GII.4 Sydney 2012 was predominant genotype followed by GII.2 in 2017–2019. Rotavirus A was detected in 88% and 81% of samples with the maximum concentrations of 5.7 and 6.3 log<sub>10</sub> copies/L in dissolved form in respective seasons. Norovirus GII was more frequently detected in 0.45–10- $\mu\text{m}$  suspended forms compared to dissolved form, while rotavirus A was predominant in dissolved form, indicating that rotavirus A was less associated with suspended solids in the surface water samples compared to norovirus GII.

We also investigated the present forms of pepper mild mottle virus (PMMoV), recently proposed as a performance indicator of virus removal in drinking water treatment processes, and found that PMMoV was predominant in dissolved form. Our observations provide important implications for understanding the behaviors of pathogenic viruses and a candidate indicator in environmental waters.

## **POP 22     Assessment of human norovirus viability in seawater**

Kata Farkas, Shelagh K Malham, Lewis Le Vay

Bangor University

Agricultural, industrial activities and urbanisation lead to the microbial pollution of the aquatic environment, including recreational water bodies, shellfish harvesting areas, drinking and irrigation water. This contamination results in an increasing number of waterborne and foodborne outbreaks. Amongst waterborne pathogens, norovirus is the lead cause of viral gastroenteritis globally. In order to reduce environment-associated illnesses, the fate and survival of norovirus in water should to be better understood. The major limitation of that is the lack of quantitative norovirus culturing technique that can be used in environmental investigations.

The aim of this study is to estimate human norovirus viability in seawater using integrity assays and indicator viruses. We assess norovirus survival in seawater using direct qRT-PCR and capsid integrity assay and correlate the results with the decay rates of culturable surrogates, such as human adenovirus, murine norovirus, crAssphage and MS2 bacteriophage. Our preliminary findings suggest that the capsid integrity assay, using porcine gastric mucin (PGM), gives good indication on norovirus concentrations and that human norovirus and adenovirus decay rates are similar in the marine environment.

The PGM assay is a rapid and straightforward method, which can be implemented in existing protocols using RT-qPCR and can be easily standardized. The routine use of the PGM assay would enable the better estimation of norovirus risks. Furthermore, the use of a reliable indicator (e.g. adenovirus or crAssphage) would enable the accurate tracking of viral contamination in water used for recreational activities, shellfish harvesting or irrigation. Hence, risks can be better assessed and public health may be improved.

## **POP 23     Limits of survival of *Mycobacterium* species in Water distribution systems**

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*Mycobacterium* encompasses more than 150 species, including pathogens and saprophytes, of which more than 100 species are termed non-tuberculous mycobacteria.

Mycobacteria are ubiquitous being distributed in a wide variety of natural and man-made reservoirs, many of which are shared by both humans and animals, namely lakes, rivers, soil, water distribution systems, among others.

In this work, the behavior of nontuberculous mycobacteria when faced with adverse conditions, particularly those to which they are subjected during disinfection processes in water distribution systems were tested. Isolated species of nontuberculous mycobacteria were challenged for their resistance to temperature, pH, chlorine and nutrient starvation.

It was observed that most of the studied species have a highly survival and growth rate when compared to other microorganisms, with a temperature growth window of 8 to 44 °C, pH of 0-14 and chlorine concentration of 1 to 10 ppm. In addition, the responses of the studied species under nutrient starvation were studied, observing that the cells entered a latent state characterized by non-morphological changes, maintenance of the concentration of cells as measured by qPCR (UG/mL) and fivefold decrease of intracellular ATP level (pmol/mL).

The obtained results demonstrated a great resistance of the isolated species to the different studied stress parameters, indicating that the isolated species presented remarkable adaptation strategies when they were exposed to extreme factors. The ability of the studied mycobacteria to adapt to extreme limits of growth justifies, in part, the maintenance and persistence of these species in water distribution systems. Further studies of resistance and disinfection of mycobacteria in water distribution systems are being performed to address the efficient removal of these bacteria from these settings.

#### **POP 24 Decay comparison of bacteriophages pp7 and p22 in water matrices, under different environmental conditions: detection by culture-based methods and qPCR**

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Virus behavior in water is usually influenced by different factors. The presence of solid particles is quite significant since they confer protection to them but this will depend on particle type, size and concentration, physicochemical properties of virus, ambient conditions, among others. The aim of this work was to compare the persistence of two phages used as RNA and DNA virus models (pp7 and p22) under different ambient conditions. A group synthetic water matrices were prepared in 500 ml-glass beakers using Phosphate Buffer Solution (PBS). A smallest particle size found (<0.074 mm) in river bed was chosen and added in each beaker in two concentrations: 5 and 0.5 g/l (high and low respectively). Then, stocks of bacteriophages pp7 (*Pseudomonas aeruginosa*) or p22 (*Salmonella*) were prepared and spiked in known concentrations in different water matrices. Beakers with virus but without particles were used as controls. Two temperature (25°C and 12 °C) and two conductivities values (130 and 1300 µS/cm) were selected to simulate ambient conditions. Water samples were taken from the surface of each beaker to evaluate virus disappearance over time. After a while, resuspension was done in all matrices and a last sample was removed and analyzed. Turbidity decay was also measured. Virus detection was done by plate count and qPCR. Kinetic parameters were calculated throughout a first-order decay model. Results showed that virus persistence was greater at low temperature, high conductivity and low solids particle concentration in water (measured through both plate count and qPCR). The persistence of the p22 phage was greater than pp7 in all the environmental conditions evaluated, being more marked for nucleic acids detection. Phage count did not increase after resuspension. Knowing virus persistence in water is important as it will allow a better understanding of the health risk involved.

#### **POP 25 Detection of a microbial source tracking marker by isothermal helicase-dependent amplification and a nucleic acid lateral-flow strip test**

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Over the last decades, PCR-based methods have been developed that can identify the sources of faecal pollution in environmental waters. These microbial source tracking (MST) methods are powerful tools to manage water quality and support public health risk assessment. However, their application requires specialized equipment and trained personnel that are usually not available in laboratories performing

routine water quality monitoring. To expand the applicability of molecular MST tools, we have developed a novel molecular amplification and detection approach that combines helicase-dependent amplification with a nucleic acid strip test (HDA-strip). As a proof-of-concept, we adapted primer and probe sequences of a well-established quantitative PCR (qPCR) assay to detect the ruminant-associated *Bacteroidetes* 16S rRNA marker BacR. The developed HDA-strip assay only requires a heating block to amplify the BacR marker. Likewise, results are easy to read and interpret by non-experts. Following amplification at a constant temperature, the reaction mixture is simply dropped onto the test strip. After 15 min, test results are displayed via the presence/absence of colored lines based on an on-strip colorimetric reaction, which relies on marker-specific hybridization probes. The entire assay can be performed in two hours and, unlike its qPCR counterpart, without the need of high-end instruments and extensive practical training. Furthermore, the BacR HDA-strip assay achieved almost identical results in head-to-head performance tests with the BacR qPCR reference, in which we investigated source-sensitivity and source-specificity, the analytical limit of detection, and the sample limit of detection. We conclude that the HDA-strip assay provides a tool complementary to qPCR methods (regarding equivalence in source-specificity and sensitivity, as well as detection limits) but without the current limitations of cost and technical complexity. Even though this approach only yields qualitative results, it can pave a way for future simple-to-use MST screening tools.

## **POP 26      Source tracking of microbial faecal pollution along a 2600 km stretch of the River Danube**

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The microbial faecal pollution of rivers severely impacts human activities that rely on appropriate river water quality. Thus, detailed knowledge of the extent and origin of microbial faecal pollution is crucial for watershed management activities to maintain safe water use. In this study, the microbial faecal pollution levels were monitored by standard faecal indicator bacteria along a 2,600 km stretch of the Danube, the world's most international river, as well as the Danube's most important tributaries. To track the origin of faecal pollution, host-associated *Bacteroidetes* genetic faecal marker qPCR assays for different host groups were applied. The spatial resolution analysis was followed by a time resolution analysis of faecal pollution patterns over one year at three selected sites. In this way, a comprehensive faecal pollution map of the total length of the Danube was created, combining substantiated information on both the extent and origin of microbial faecal pollution. Within the environmental data matrix for the river, microbial faecal pollution constituted an independent component and did not cluster with any other measured environmental parameters. Generally, midstream samples representatively depicted the microbial pollution levels at the respective river sites. However, at a few, somewhat unexpected sites, high pollution levels occurred in the lateral zones of the river while the midstream zone had good water quality. Human faecal pollution was demonstrated as the primary pollution source along the whole river, while animal faecal pollution was of minor importance. This study demonstrates that the application of host-associated genetic microbial source tracking markers in concert with the traditional microbial faecal pollution monitoring significantly enhances the knowledge of the extent and origin of microbial faecal pollution patterns in large rivers. It constitutes a powerful tool to guide target-oriented water quality management in large river basins.

**POP 27      *Salmonella* Diversity and Distribution in Irrigation Ponds, Irrigation Systems, and Produce on Farms in Southern Georgia**

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To determine potential risks of irrigating commercial crops with surface water, this study investigated the presence and diversity of *Salmonella* in irrigation ponds ( $n=24$ ), wells ( $n=5$ ), irrigation distribution systems ( $n=65$ ), and on produce ( $n=65$ ) from four farms in southern Georgia. *Salmonella* was detected in water and produce samples, albeit in low concentrations (<1 MPN/100 ml water sample and <2 MPN/produce sample, respectively). We detected *Salmonella* in eight (33.3%) pond, 16 (24.6%) irrigation system, one (20%) well, and two (3%) produce samples. *Salmonella* isolates ( $n=37$ ) were typed using serotyping, pulsed-field gel electrophoresis (PFGE), and whole genome sequencing. Eight distinct serovars occurred in the samples, four of which (Newport, Javiana, Muenchen, and Saintpaul) are consistently isolated from clinical samples in Georgia. Sixteen distinguishable PFGE patterns were detected. No PFGE pattern co-occurred in all three sample types from the same farm (source waters, irrigation water, produce), but seven patterns were detected in ponds and irrigation systems from the same farm. On one farm, one pattern was detected in the irrigation system and on produce, but samples were collected two months apart. All but two PFGE patterns detected in this study also occurred in the Centers for Disease Control and Prevention's PulseNet Database. Whole genome sequencing uncovered additional diversity, and revealed that study isolates clustered with human isolates across several continents. We conclude that our study in southern Georgia resulted in the isolation of low concentrations of clinically relevant *Salmonella* in surface water irrigation ponds, irrigation distribution systems, and on commercial produce.

**POP 28      Are septic systems the source of human fecal contamination in private wells in rural Pennsylvania?**

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In Pennsylvania (PA), USA, approximately 3 million people rely on domestic well water for drinking. To date, few studies have examined the presence of pathogens in groundwater, and those that have, focus on spatial variability rather than temporal variability of pathogens in wells. The goal of this study was to investigate the temporal variability and influence of rainfall on private groundwater supplies in PA.

Five wells were selected for study in rural PA. In 2016, samples were collected every two weeks from 5 wells ( $n=34$ ), and in 2017 samples were collected weekly from 3 wells ( $n=72$ ). Samples were analyzed for: general water chemistry; *E. coli* and total coliforms; real-time qPCR for human sewage markers and viral and bacterial pathogens.

All wells were positive for human *Bacteroides* on 2 to 8 sampling events ( $n= 26$ ). Concentrations ranged from 0.63 gc/L to 3454 gc/L. One well was positive for human polyomavirus (2.3 gc/L). All five wells were positive for pepper mild mottle virus on one or more occasions ( $n=9$ ; 2.1-585.5 gc/L). No wells were positive for the other pathogens monitored.

Cumulative rainfall 8-14 days prior was related to the concentration of human *Bacteroides* ( $p=0.008$ ) and coliforms ( $p=0.069$ ), respectively in the wells. No relationship existed between *E.coli* and rainfall. In robust regression analyses, increases in conductivity, turbidity, and fluoride as well as decreases in nitrate were highly predictive of increases in coliform concentrations in the wells ( $p <0.0001$  to  $<0.05$ ).

To further investigate the source of contamination, in 2018,, fluorescein dye was flushed down the household toilets, while the households' well water was run continuously through a fluorometer. Dye was detected by the fluorometer at all 3 wells studied in 2018. The results confirm that household septic systems are impacting private groundwater supplies.

**POP 29 A multivariable approach to and correlation analysis of *Bacteroidales* and crAssphage genetic markers for microbial source tracking in Thailand**

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Water quality impairment is a problem of increasing concern worldwide. The origin of fecal water pollution can be traced by detecting host-specific gastrointestinal microbiota excreted with feces. This growing area of research and development is called microbial source tracking (MST). However, the abundance and distribution of gut microbial communities differ in different geographical regions and by host, affecting the performance of MST markers. This study evaluates the performance of 17 molecular markers for *Bacteroidales* (including universal markers and human-, swine-, and cattle-specific ones), as well as human-specific crAssphage, for MST in Thailand. An endpoint PCR technique was used to screen candidate assays and provide sensitivity, specificity, accuracy, and positive/negative predictive values. The detection limits for each assay were also determined. Among the markers tested in this study, the best-performing PCR assays for human-, swine-, and cattle-specific sources were crAssphage, Pig-2-Bac, and Bac3, respectively. These were further evaluated with a qPCR platform. Universal fecal markers that showed equally good performance were BacUni and GenBac. The latter was selected for further characterization with a corresponding qPCR platform. Both endpoint PCR and qPCR assays were used for source identification in river water samples along Thailand's 325-kilometer Tha Chin river, which historically has the most coliform bacteria pollution. This study provides a selection and validation of the best performing assays for host-specific MST markers, their application in Tha Chin water samples, and a correlation with precipitation, land use, and other water quality parameters. As part of this research project, two policy-related documents were produced: 1) MST guidelines for laboratory personnel; and 2) a policy brief for MST application in Thailand.

**POP 30 Event-based survey of urban and agricultural drinking water sources: assessing fecal peak contamination using microbial and chemical source tracking tools**

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A major hazard to drinking water safety is presented by intense rainfall and snow melt events, where large quantities of fecal material may be discharged from storm or sewage overflows or washed from the catchment into the water source. This study utilized microbial source tracking (MST), chemical source tracking (CST) markers, enteric viruses, water quality indicators (*E. coli*, turbidity) and hydrometeorological data (flowrate, precipitations) to assess the vulnerability of two urban and one agricultural drinking water intakes (DWIs) and identify sources of fecal contamination. Water samples were collected during background and multiple snow and rain events and analyzed for human, bovine

and porcine mitochondrial DNA markers (mtDNA) and for human-specific *Bacteroidales* HF183 marker along human wastewater micropollutants (WWMPs) including caffeine, carbamazepine, theophylline and acetaminophen. Event-based monitoring duration varied between 18h to 5 days and revealed that precipitations events triggered the microbial and chemical peak concentrations. Dynamics of *E. coli*, HF183, and WWMPs were similar and concentrations generally varied over one order of magnitude during each event. Elevated human-specific marker levels during events accurately identified that the urban DWIs were impacted by treated and untreated discharges from upstream water resources recovery facilities (WRRFs). In the agricultural catchment, the occurrences of mitoHu (human), mitoBo (bovine), mitoPo (porcine) DNA were 44%, 78%, and 44% respectively, while HF183 tested negative for all the samples. mitoBo qPCR concentrations showed high levels of cattle fecal pollution following rain events. This reflected rainfall-induced loading of cattle-derived fecal pollutants to DWI from upstream non-point sources. Additionally, genotyping of rotaviruses pointed towards porcine sources of fecal pollution, thereby confirming the findings with the mitoPo marker. This study demonstrated the value of high risk event sampling strategy and using both MST and CST methodologies to more reliably assess sewage and livestock contamination in drinking water sources.

### **POP 31 Validation of an avian MST toolbox to identify bird fecal pollution in environmental waters in France**

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Environmental water quality can be impacted by bird droppings due to the presence of bacterial pathogens.

The aim of this study was to evaluate the performance characteristics of Microbial Source Tracking (MST) markers targeting birds by screening avian ( $n= 345$ ) and non-avian ( $n= 308$ ) fecal samples from two contrasting regions in France (Brittany and Aquitaine). Both fecal collections contain a range of host samples. Specifically, the Brittany collection contains shorebird birds in addition to poultry litter samples, while the Aquitaine collection contains domestic birds breed outdoors and wild birds.

The bacterial qPCR markers quantified in the samples include two general bird feces markers (*Helicobacter* spp. associated GFD and *Lactobacillus* associated AV4143), one poultry feces marker (Firmicutes associated AV43), one poultry litter marker (*Brevibacterium* associated LA35) and one gull feces marker targeting *Catellicoccus marimammalium*, (Gull2 or Gull4).

When used in combination both general bird markers allow for identification of bird fecal impacts in 67.4% and 73.6% of avian samples in Brittany and Aquitaine, respectively. The general bird markers had a specificity higher than 96%. The LA35 marker was confirmed to be an efficient poultry litter marker in Brittany with 87.1% positive in poultry litter samples but only 29.7% in poultry feces. The AV43 marker was poultry associated and detected in 70.2% of the Brittany and 66.7 % of the Aquitaine samples. The gull markers (i.e. Gull4 in Brittany and Gull2 in Aquitaine) were frequently detected in gull feces in both regions (81.8% and 100%, respectively). The frequent detection of Gull4 marker in seabirds, suggests it is a better shorebird marker than a gull marker in France.

The application of these MST markers on bird-impacted and non-impacted water samples show a relatively high occurrence of Gull2 and AV43 markers in bird-impacted waters. The MST markers selected in this study constitute an efficient MST toolbox to evaluate avian pollution in France.

**POP 32 Faecal coliform and *Salmonella* spp. in household drinking waters in Dhaka city: an overlooked health hazard**

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In a developing country setting including Bangladesh, the supply of clean drinking water to household is often compromised due to old or broken underground water and sewage pipes, although the water is generally deemed to be safe at the treatment plants or supply-origin. Presence of pathogenic microorganisms in the household water poses risk of enteric diseases including *Salmonella* infections, which is endemic in Bangladesh. In an attempt to assess the health risk of microbial contaminants in piped household water supply, we analysed tap water samples collected from 144 households across 11 different zones of Dhaka city. Water samples were analysed for faecal coliforms (FC) by standard membrane-filtration and culture whereas the presence of *Salmonella* spp. was ascertained via *invA*-PCR after primary-enrichment. Of the 356 tap water samples tested, only 27% were free of FC whereas the remaining 73% contained FC at various concentration; FC counts were 1-30, 31-100, and >101 cfu/100mL for 39%, 14%, and 20% samples respectively. Further, 14% of tap waters were tested PCR-positive for *Salmonella*. Most samples (94%) that were positive for *Salmonella* were simultaneously positive for >30 cfu of FC per 100 ml. In a parallel Laboratory experiment, when autoclaved tap water samples were spiked with a strain of *S. Typhi*, the bacteria remained culturable for up to 5 days, suggesting that microbial contaminants in the supply-water could persist long enough to pose a health risk. While guidelines for drinking water allow no FC, these results reflect high level of faecal contamination in tap-water in Dhaka, indicating serious health risk, and highlights the necessity of routine monitoring of supply-waters at household-level, as well as devise ways to mitigate the risk of waterborne infections.

**POP 33 Higher occurrence of norovirus GII during summer and autumn in the southern part of Lake Biwa, Japan**

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Human noroviruses are a major cause of gastroenteritis, and have been frequently detected in water environment. Discharges from sewage treatment plants (STPs) are considered to be a source of the norovirus contamination. However, their occurrence in lakes, and correlation between their concentrations in there and in discharges from STPs haven't been fully studied yet. To understand factors causing the concentration fluctuation of norovirus GII (GII-NoV) in the lake, we quantitatively monitored GII-NoV every month in the southern part of Lake Biwa in Japan from July 2014 to December 2018. In parallel, we measured the concentration in the influent and the final effluent of three STPs which discharges the final effluent to the lake. For the concentration, we used the negatively charged membrane method coupled with RT-qPCR. Furthermore, we collected data of the amount of the primary effluent that bypassed biological treatment processes during rainfall events in one of the STPs. We found the concentration in the influent fluctuated from  $2.79 \times 10^3$  to  $6.00 \times 10^6$  copies/L, and was more abundant in winter.

The concentration in the final effluent fluctuated from  $1.67 \times 10^2$  to  $1.37 \times 10^4$  copies/L, which showed the same seasonal change as the influent. However, the concentration in the lake fluctuated differently; it varied from  $1.34 \times 10^2$  to  $5.08 \times 10^5$  copies/L, and increased in late summer and autumn when a large amount of the primary effluent bypassed the biological treatment processes due to heavy rainfall events. In particular, in 2016 and 2017, the highest concentration of the lake was higher than that of the final effluent, and showed the positive correlation with the amount of the discharged primary effluent. These findings indicate that the bypassing of the primary effluent in the rainy events may be a cause of the norovirus increase in the lake.

#### **POP 34      The presence of water-associated coliforms in our drinking water and their effect on drinking water quality**

De Maeyer Katrien, Huysman Koen, Goos Karel, Bielen Paul

Pidpa

According to the drinking water legislation, coliforms are not allowed in drinking water. Total coliforms are a heterogeneous group, making it often difficult to define the significance of encountering this indicator parameter.

As a drinking water company, we have experienced the colonization of a part of our distribution network, derived from a specific filtration step in one of our water production centers (WPC), with a certain type of coliform. More specifically it concerns a *Serratia* species (based on 16S rRNA gene sequencing), which can not yet be identified with our current routine identification systems (e.g. Maldi-tof MS, biochemical identification). As our Flemish legislation allows to discriminate in the corrective actions depending on the coliform species that has been detected, a better characterisation of this strain imposes itself.

Different isolates of this strain were collected throughout a few months in different regions of the distribution network and the WPC itself. A dereplication study by Maldi-tof MS revealed a very high similarity between all those isolates, in contrast with a group of isolates of the species *Serratia fonticola* which showed a lot more variation across different locations of origin. Apparently we are facing a *Serratia* species that resides very well in drinking water systems, that is not yet implemented in databases of identification systems or is possibly not yet described at all.

Even though this species probably not poses a risk for human health, it interferes with the current detection techniques for total coliforms and more insight is needed in how this kind of water-associated coliforms can be overcome. Recent findings suggest that they might start to flourish on filters in the treatment process with no specific directed microbial activities (e.g. iron oxidation or nitrification).

## **POP SESSION WEDNESDAY**

### **POP 35 UV LED Water Disinfection: Validation and Small System Demonstration Study**

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Disinfection by ultraviolet (UV) light via non-mercury light-emitting diodes (LEDs) may be a sustainable solution for drinking water disinfection in small systems. The world's first commercial UVC LED water disinfection reactor (the PearlAqua by Aquisense) was studied over a year-long demonstration test, and performance was compared side-by-side with an existing chlorination system at a small water treatment plant in Colorado using slow sand filtration. The UVC LED disinfection system was initially validation tested using MS2 bacteriophage inactivation over a range of flowrates and water UV transmittances. During the field study over the year, UVT, turbidity, temperature and pH were measured daily. Bi-weekly samples were tested for adenosine triphosphate (ATP), total coliform and *E. coli*(TC/EC), and total and dissolved organic carbon (TOC/DOC). Once every three months (quarterly), the disinfection performance of the UV reactor was evaluated by MS2 challenge testing along with measures of total dissolved solids (TDS), Alkalinity, Hardness, Nitrate/Nitrite, and inorganics (including Br). Disinfection byproducts were measured twice over the year. The field study indicated the resilience and disinfection effectiveness of the UV LED reactor equivalent to the parallel chlorination system, operated continuously with zero maintenance, with an estimated yearly electrical cost of less than \$25 to disinfect water flowing at 0.5 lpm. The system consistently performed at an MS2 bacteriophage reduction equivalent dose (RED) of at least 40 mJ/cm<sup>2</sup>. The longitudinal evaluation of the flow-through UV LED system provides data necessary for practical operation, design improvements, and scale-up, allowing faster adoption in the future. Future work should consider scale-up to meet flow demands of municipal systems, and modifications for lower UVT water such as wastewater and reclaimed water.

### **POP 36 Development of proteome analysis of viral proteins for evaluation of damage by disinfectants**

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Presence of human infectious enteric viruses in environmental waters around the world is becoming clarified. Inadequate disinfection will lead to an outbreak of waterborne infectious diseases. Unlike bacteria, disinfection effects such as chlorination have limitations on viruses, so it is necessary to introduce the multi-barrier concept using some physical and chemical treatments, and to consider multifaceted inactivation of the virus. Estimations of the inactivation mechanism of viruses in disinfection processes are important for optimizing combinations of them.

Several studies have reported that virus proteins can be separated by gel electrophoresis and the mass of each protein is measured by mass spectrometry after in-gel digestion. However, this electrophoresis-based method consumes much time to analyze the mass of viral protein. On the other hand, mass spectrometry-based method without using the gel electrophoresis has been developed. The objective of this study is development of analysis method of viral protein damage by mass spectrometry to understand the virus inactivation mechanism associated with each disinfection process.

Bacteriophage MS2 was used as a vial surrogate of enteric viruses in this study. First, purification methods of virus samples were investigated. Consequently, combination of differential centrifugation and isopycnic centrifugation was an effective method for removal of *Escherichia coli* protein and other contaminants. Next, the analytical conditions for peptides including MS2 protein by liquid chromatography tandem mass spectrometry (LC-MS/MS) were determined using the database of amino acid sequence of MS2 protein and Skyline. One hundred and eight multiple reaction monitoring (MRM) transitions were selected from 2157 MRM transitions for monitoring of peptides including MS2 protein. Finally, we evaluated protein damages by chlorination, ultraviolet (UV), ozonation and UV/H<sub>2</sub>O<sub>2</sub> by LC-MS/MS. As a result, some pieces of detailed inactivation mechanism were proposed.

**POP 37      Evaluation of 20L transparent containers for household solar water disinfection (SODIS) of drinking water in resource-poor environments: The WATERSPOUTT Project.**

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Solar water disinfection (SODIS) is an appropriate technology for treating drinking water in developing communities, as it is effective, low cost, and easy to use. Nevertheless, uptake is low due partially to the burden of using small volume polyethylene terephthalate bottles (1.5-2 L). A major challenge is to develop a low-cost transparent container for disinfecting larger volumes of water. This study examines the capability of 20 L transparent PET jerrycans & polypropylene (PP) buckets to be used for the purposes of SODIS using three waterborne pathogen indicators: *Escherichia coli*, MS2-phage and *Cryptosporidium parvum* oocysts. We examine the factors that were taken into consideration during the design phase; UV transparency and material aging under natural and accelerated sunlight, microbicidal efficacy under real sunlight conditions and challenges facing implementation in the context of low-income environments in rural Ethiopia (jerrycan) and rural Malawi (bucket). Similar inactivation kinetics were observed under natural sunlight for the inactivation of all three organisms in well water using 20 L -buckets compared to 1.5 L PET bottles. Both materials were exposed to natural and accelerated solar aging (ISO-16474). UV transmission of the 20 L-buckets remained stable and with physical integrity even after the longest aging periods. This work shows that transparent 20 L PP buckets represent a good alternative to the PET bottles usually employed for SODIS. The 20L PET jerrycans have similar microbicidal capacities against bacteria and viruses as 2L PET bottles but have poorer capacity to inactivate *C. parvum* oocysts.

**POP 38      Effect of Chloramine Residuals, Nitrification Stages, and Bulk Water pH on Occurrence and Growth of Opportunistic Pathogens**

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Spread of opportunistic pathogens through the water distribution systems has been a growing concern, yet the factors influencing their proliferation in chloraminated water distribution systems are largely unknown. This study investigated the impact of chloramine residuals, water pH and nitrification stages on occurrence and growth of opportunistic pathogens (*Legionella longbeachae*, *Legionella pneumophila*, *Mycobacterium avium*, and *Pseudomonas aeruginosa*), broader genera (*Legionella* and *Mycobacteria*), and two amoeba hosts (*Acanthamoeba* spp. and *Hartmanella vermiformis*). Four sets of the lab-scale systems, each system comprising three reactors connected in series were operated with

pH 7.6, 8.4 and 9.0 and initial chloramine residuals 2.6 mg/L and 3.8 mg/L. In each set of the reactor system, chloramine residuals decreased along the reactors and reached below the detection limit in the last reactor. Similarly, nitrification progressed from no nitrification to the severe nitrification along the reactors. MiSeq and qPCR techniques were employed to classify bacterial community and to quantify target gene copies number. *Mycobacteria*, *Legionella*, and *Pseudomonas* naturally colonized in all reactor systems whereas *Legionella pneumophila*, *Legionella longebeach*, and *Hartmanella vermiciformis* were not detected. *Mycobacteria avum* were detected in the system operated with pH 7.6 and 8.4 whereas *Acanthamoeba* spp were detected in pH 8.4. Comparing to other genera, *Mycobacteria* were highly dominated in all systems. Although the relative abundances of *Mycobacteria* were high in high chloramine residuals (>1.0 mg/L) and *Legionella* in low chloramine residuals (<0.5 mg/L), the gene copies number of *Mycobacteria*, *Legionella* and *Pseudomonas aeruginosa* increased with decreasing chloramine residuals and/or with the progress of the nitrification stages. The significant impact of pH alone on the population of opportunistic pathogens was not observed, but the combination of pH and chloramine residual influenced their growth. This study shows that by changing the operating conditions of the water distribution system, opportunistic pathogens could be controlled.

#### **POP 39     Fate of antibiotic microbial resistance during ozonation of wastewater treatment plant effluent**

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Wastewater treatment plants are considered as an important contributor to the spread of antibiotic microbial resistance in urban aquatic environments. Ozone is known to remove trace organic chemicals, but inconsistent results have been reported on its efficiency for removal of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs).

The objective of this study was to systematically investigate the elimination of target indicator bacteria (*E. coli*, *Pseudomonas aeruginosa* and intestinal enterococci) and their ARGs (Sul1, BlaVIM1 and VanA) by ozonation for different operational conditions. Ozone reaction kinetics were determined in bench-scale ozonation experiments performed in secondary effluent (SE) and in buffered ultrapure water containing cultures of susceptible and resistant bacteria. The inactivation of bacteria was evaluated by cultivation methods and monitoring of cell membrane integrity. Quantification of total cell concentration (16S rRNA) and ARGs was performed by quantitative polymerase chain reaction (qPCR), while using different length of 16SrDNA primers to investigate the gene reduction according to their amplicon lengths (202 and 457 bp).

Results confirm a very fast inactivation without significant difference between resistant and non-resistant bacteria but the ozone susceptibility vary between individual species. A rapid decline of cell integrity was observed within the first 10 seconds (corresponding to 0.015 mg O<sub>3</sub>.L<sup>-1</sup>.min), making this method not suitable to track inactivation by several orders of magnitude. ARGs and 16SrDNA gene copies showed different susceptibility to ozone treatment, e.g. a 2 log removal of VanA (gene copies/100mL) required almost two times higher ozone exposure than 16S rDNA, raising the question of suitability of qPCR to assess efficacy of treatment processes. Although, efficient cell inactivation >1.5 log CFU/100mL in SE was observed applying 0.32 mgO<sub>3</sub>.L<sup>-1</sup>.min (or an ozone exposure of 0.75 mgO<sub>3</sub>.mgDOC<sup>-1</sup>), maximum 1 log reduction in number of gene copies was achieved independently on the measured length of the amplified gene.

**POP 40 Household-level water treatment practices for removing faecal organisms in drinking water: lessons learnt from Dhaka**

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In developing countries including Bangladesh, leakage in underground water and sewage pipes often compromise the supply of clean drinking water. Therefore, boiling and filtration methods are commonly practiced for water-treatment in the households. While the water-treatments should ideally remove all faecal organisms from the raw waters, scarcity of household-level surveillance data leaves a critical knowledge gap regarding the level of resultant health risk. In an attempt to address this gap, we analysed treated water samples collected from 114 households of Dhaka city. Standard membrane-filtration and culture was used for enumeration of faecal coliforms (FC) whereas the presence of *Salmonella* spp. was ascertained via *invA*-PCR after primary-enrichment. Of the 274 samples tested, 51% were free of FC; the remaining samples produced variable FC counts of 1-30, 31-100, and >101 cfu/100mL for 38%, 9%, and 2% samples respectively. Additionally, 2% of treated waters (all having FC counts of >31 cfu/100mL) were tested PCR-positive for *Salmonella* after primary enrichment. FC-removal efficiency for boiling (practiced in 37% households) and filtration (in 29% households) did not differ significantly from each other; both frequently yielded FC counts. While poor performance of household filtration units remains a concern, occasional detection of FC in samples that underwent boiling followed by filtration (practiced in 23% households) suggests that chances of post-treatment contamination (possibly due to inadequate personal or utensil hygiene) cannot be overlooked. Regardless of the actual sources, presence of faecal organisms in treated ready-to-drink waters might pose a potential health risk for over 20 million population of Dhaka city. This study highlights the necessity of monitoring the performances of treatment methods, identify challenges to ensuring safe waters, and devise ways to mitigate the risk of waterborne infections at household-level, where raw water can be contaminated with faecal organisms.

**POP 41 Chitosan Coagulation-flocculation Pre-treatment Increases Virus and Bacteria Reductions by Intermittent Slow Sand Filtration of Drinking Water**

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Sand filtration water treatment has limited effectiveness in reducing bacteria and viruses, unless operated at low flow rate to promote biological activity, as in slow sand or biosand filters, or preceded by chemical coagulation to flocculate bacteria and viruses. Alum and iron salts are used typically for chemical coagulation but are difficult to dose optimally and therefore rarely used for POU treatment. As an alternative, chitosan, a natural, high molecular weight, organic, non-toxic polymer derived from chitin, was evaluated for reduction of viruses and bacteria by slow sand or biosand filters. Chitosan effectiveness in removing coliphage MS2 indicator virus and *E. coli* bacteria from natural surface water was determined in miniature (2.5 cm diameter by 16 cm deep), intermittent flow slow sand filter columns over a period of 57 days. Two grain sizes of sand filter media ( $d_{10} = 0.24$  mm and 0.5 mm;  $d_{160}/d_{10} = 1.4$  both) and 3 doses of chitosan acetate were used (3, 10 and 30 mg/L). In water not treated with chitosan, average reductions of MS2 and *E. coli* were <1 log<sub>10</sub>. Average log<sub>10</sub> reductions of MS2 and *E. coli* were significantly improved by chitosan coagulation-flocculation prior to sand filtration. At the optimum chitosan dose of 10 mg/L, average log<sub>10</sub> reductions of MS2 and *E. coli* were >4 log<sub>10</sub> and turbidity was reduced to <1 NTU. These observed reductions of MS2 and *E. coli* by chitosan coagulation and slow sand filtration meet the World Health Organization 2-star "protective" performance targets of 3 log<sub>10</sub> for viruses and 2 log<sub>10</sub> for bacteria for household water

treatment. Chitosan coagulation-flocculation followed by shallow bed sand filtration has great potential as a practical and simple technology to achieve microbially safe community and household drinking water and deserves further performance validation in lab and field studies.

#### **POP 42 Free Chlorine Sensitivity of Rotavirus Exposed to Repeated Disinfection and Bottleneck Events**

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Free chlorine inactivates gastroenteritis viruses but its effectiveness can vary along with a change of population structure of gastroenteritis viruses. A population of RNA virus displays higher genetic diversity owing to high mutation rate. The genetic diversity of a viral population is also affected by natural selection (advantageous genes are fixed in a population) and bottleneck effect (genes are randomly selected for next generations) in a life cycle of gastroenteritis viruses. In this study, we hypothesized that free chlorine disinfection could act as selection pressure on gastroenteritis viruses. We used rhesus rotavirus (RRV) as a model gastroenteritis virus, and investigated the sensitivity change of RRV to free chlorine along with serial passages with dilution and free chlorine disinfection. As a result, we found a decrease of free chlorine sensitivity (0.5 log-reduction at Ct-value=1.9) after the third-passage in a lineage while a less-sensitive population in another lineage recovered the sensitivity (1.0 log-reduction at Ct-value=1.9) after the forth-passage. RRV population structure could be changed mainly by a bottleneck effect, so that the sensitivity of an RRV population to free chlorine was randomly changed. We also found that less-sensitive populations had higher nucleotide diversity while no significant differences in a cell-binding and specific growth rate were observed between five less-sensitive and fifteen sensitive populations (p-value: 0.20 and 0.06). These results indicate that less-sensitive mutants to free chlorine can accidentally appear from a deep layer of swarm composed of minor mutants, resulting in an increment of genetic diversity. Such mutants immediately disappear from a population or turn back to a mutant swarm during the following passages, which results in a stochastic change of free chlorine sensitivity as a population. This study suggests that inadequate disinfection of gastroenteritis viruses in water may lead to a sporadic appearance of rare but disinfection-resistant mutants.

#### **POP 43 Variability of free chlorine resistance among Human Enterovirus B strains leads to insufficient inactivation in drinking water**

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Viruses are prone to have high mutation ratio and evolve rapidly. Recent studies showed that currently circulating viruses are diverse and genetically different from lab strains (Lukashev et al. 2017. *J. Gen. Virol.*). This suggests that lab strains might be no longer the representative of current viruses although it is widely used for water treatment experiment. For example, a field isolate of coxsackievirus (CV) B5 exhibited 6 times higher free chlorine (FC) resistance than the lab strain (Meister et al. 2018., *ES&T*). We aimed to determine the variability in FC resistance of enteroviruses and to evaluate its effect on inactivation rate in drinking water.

We collected 17 field isolates of *Human Enterovirus B* (CVB3: n=7, CVB4: n=3, CVB5: n=7) from wastewater influent from 2006-2015 in Toyama, Japan, which may include more diverse viruses than the previous study (Meister et al. 2018). Then, these viruses were propagated by BGM cell and purified by CsCl density gradient, followed by test for FC resistance, where FC was applied at 0.3 – 0.4 mg/L in 10 mM phosphate buffer at 22 °C.

Upon microbial risk management, the FC inactivation profile is assumed to be homogeneous within the same serotypes or species. However, the differences in FC resistance were as great as 7 times within the same serotype and 10 times among *Human Enterovirus B*. Supposing that the examined viruses are mixed in water sources evenly, the variability leads to tailing of inactivation. For instance, to inactivate CVB5 by 1 and 4-log10, the mixture requires 2.5 and 5.2 times larger CT than CVB5 (lab strain), respectively. Interestingly, CVB4s showed less resistance than CVB5s. Consequently, the variability of disinfection resistance within the same serotypes or species should be included in the estimation of log reduction value by FC.

#### **POP 44 Enhancing effectiveness and acceptability of chlorine-based POU disinfection applied to drinking waters in rural communities in Brazil**

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Most households in rural settings, especially in low-income communities, rely on alternative water sources (e.g., rainwater). The inappropriate handling and storage of water within the domestic environment can introduce enteric pathogens and make it unsafe for consumption. Despite chlorine-based disinfection being a 'cornerstone' of most municipal water treatment systems, the level of its acceptability as a household-level treatment option is very low, mainly due to taste issues relating to free chlorine residual in treated waters.

This study aimed to develop a simple and low-cost water treatment design to support the use and acceptability of household-level chlorine-based disinfection among rural communities in Brazil. The proposed 'Point-of-Use' (POU) treatment consisted of a ceramic candle filter, followed by chlorination and activated carbon filtration. Preliminary trials were undertaken to determine doses and methods for chlorine application, chlorine residual, and to identify the most appropriate activated carbon filter. The efficacy of proposed POU water treatment was evaluated in terms of reduction of *E. coli* and removal of free chlorine before consumption.

The outcomes suggested that levels of chlorine in rainwater above 0.5 mg/L can be maintained for 24 hours within the storage reservoir. Dosing household liquid bleach every time the ceramic filter is filled up seems to be the most appropriate form of chlorine application. The simple activated carbon filter device was able to effectively reduce free chlorine to non-perceptible levels. Moreover, the proposed POU water treatment was very effective (reduction > 5 log) in reducing the mean concentration of *E. coli*.

In conclusion, the POU water treatment designed for rural settings showed great potential to decrease microbial contamination to much safer levels and avoid acceptability issues due to reduction of free chlorine residual at the consumption. However, further research is necessary in order to investigate removal of more persistent microorganisms.

#### **POP 45 Removal of bacteriophage MS2 by a newly developed porous carbon material with high pore volume**

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In recent years, activated carbon produced from coal and coconut husks has been used in water purification process. Purification by activated carbon is very important to contribute to safe water supply. We developed a new porous carbon material made from rice husks with high pore volume. In this study, we investigated the porous structure of the new material to reveal the difference from various conventional activated carbons by using field emission scanning electron microscope (FE-SEM), nitrogen adsorption and mercury porosimetry measurements. Then, adsorption experiment was conducted for bacteriophage MS2 to evaluate removal performance.

The new material has a unique finely structured porous structure, which might attribute of surplus biomass such as rice husks including silica. In the FE-SEM observation, volumes of mesopore (2-50nm) and macropore (approx. 1µm) were much higher in the new material than in coconuts husk-based activated carbon used for conventional water purification. In addition, micro-pores (2nm or less) were also found as much as conventional activated carbons. In mercury porosimetry measurement, the total pore volume of the new material was the highest among the tested activated carbon. The removal of bacteriophage MS2 was observed above 5Log, while those of other conventional activated carbons was around 1-3Log. Moreover, log reduction value of bacteriophage MS2 was proportional to total pore volume measured by mercury porosimetry. The newly developed porous carbon material is expected to apply for not only the novel air filter element but also water purification system for safe water supply.

**POP 46      Cultivation of *Nitrososphaera viennensis*-like ammonia oxidizing archaea from tropical chloraminated drinking water distribution system**

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Nitrification is a common occurrence in drinking water distribution systems (DWDS) employing chloramination for secondary disinfection, where nitrifiers like ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) are regularly detected during nitrification episodes. Ammonia-oxidizing archaea (AOA) are usually only found when ammonia and/or residual chloramine concentrations are very low and little is known about their abundance and function in this environment. The cultivation of AOA is more challenging than that of AOB, with various strategies such as filtration and antibiotic treatment having been developed to selectively cultivate AOA over AOB. Such methods had been met with limited success, often times sterilizing the culture or reducing the growth rate of the AOA. In this study, a method was developed to help AOA outcompete AOB during enrichment, by means of chemical inhibition of AOB respiration through the use of dimethylthiourea and pyruvate, while stimulating the growth of AOA. Defined mixtures of AOA and AOB were cultivated in the presence of both dimethylthiourea and pyruvate or pyruvate only, or in the absence of either compound. The proportion of AOA increased to over 99% of ammonia oxidizers after cultivation with both compounds, up from an initial 30% AOA and 70% AOB. Heterotrophic bacteria were also present. In contrast, cultivation with only pyruvate produced cultures with an AOA content of less than 3% compared to 1% after cultivation without added compounds. This new enrichment strategy will facilitate the study of interactions between AOA and monochloramine, by selectively enriching AOA over AOB without sacrificing the growth rate to obtain a purified AOA enrichment. Future studies on the relationship between AOA and monochloramine can inform on its regrowth pattern in chloraminated DWDS.

**POP 47      Detection of *Salmonella enterica* serotype Typhi in Environmental Samples: A New Approach for Typhoid Fever Surveillance?**

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**Background:** The burden of typhoid fever in sub-Saharan Africa is high, particularly in urban areas with inadequate sanitation. To characterize *Salmonella enterica* serotype Typhi transmission dynamics in Kibera, a large informal settlement in Nairobi, Kenya, we conducted a longitudinal environmental study to detect Typhi in sewage-impacted drainage streams and vended drinking water using culture- and molecular-based methods.

**Methods:** Ultrafiltration was used to concentrate 1–100L of drainage and drinking water from four sampling sites every two months over the course of one year. Ultrafilter concentrates underwent standard culture methods to obtain presumptive *Salmonella* colonies, which were screened for *Salmonella* spp. and Typhi by a published real time PCR isolate assay. A new real time PCR assay specific for Typhi was developed. A subset (n=4) of preserved enrichment broth aliquots (N=144) was halved, and one of each pair was seeded with Typhi; the new real time PCR assay was applied to the paired aliquots to evaluate matrix effects. Targeted amplicon sequencing was used for single nucleotide polymorphism (SNP) identification of Typhi-positive unseeded enrichments.

**Results:** Presumptive *Salmonella* colonies were isolated from 60 (83%) of 72 drainage samples and 11 (15%) of 72 drinking water samples; 26 (90%) of the 29 samples screened to date are *Salmonella* spp., but none are Typhi. The new real-time PCR assay detected Typhi in the four paired seeded and unseeded enrichments. Targeted amplicon sequencing identified one SNP difference in unseeded enrichments when compared with known Typhi strains.

**Conclusions:** Environmental surveillance for Typhi can help identify sources of transmission and inform prevention and control strategies. The new real time PCR assay has potential for use for Typhi detection in complex environmental samples. Advanced molecular detection tools will be used to further evaluate preserved drainage and water samples.

#### **POP 48      Integrated water management in the Lurín catchment (Lima, Peru) – a case study for municipalities in the rural areas of the Andean mountains**

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According to the UN SDG 6, the universal access to safe drinking water and sanitation should be achieved by 2030. This is especially a challenge in prosperous regions with water scarcity, like Lima/Peru. Within the TRUST project, solutions for water supply and wastewater management are developed for the catchment of the Lurín River near Lima.

In the Lurín catchment, the seasonal rainfall is restricted to the upper parts above 2000 m altitude, leading to a pronounced seasonality of the Lurín discharge. While in the lower parts, the aquifer is the natural reservoir for drinking water, in the rural areas of the upper catchment, the population relies on surface water that is stored in reservoirs. One municipality in this region was chosen as a case study to evaluate the risks and needs of the existing drinking water supply and wastewater management.

Detailed analyses of the local water cycle, i.e. the assessment of the water resources, catchment, and infrastructure together with microbiological monitoring showed that fecal contamination of drinking water is a major concern. Detailed microbiological analyses indicate that fecal contamination is prevalent throughout the year and farm animals are a major cause for these contaminations. Furthermore, the direct use of untreated wastewater for irrigation leads to additional health risks.

On the basis of these investigations, integrated concepts for safe drinking water and sanitation have been developed and discussed with stakeholders on a local, regional and national level. It became clear, that the technological solutions have to be simple, affordable, and need to be locally adapted and accepted in order to become successfully implemented and operated in future. In order to achieve a sustainable improvement of the situation the implementation needs to be incorporated in a holistic concept strengthening local awareness, capacities, and water management institutions in addition to efficient treatment and protection of the drinking water resources.

**POP 49 Prevalence of opportunist pathogens in water sources in the Kathmandu Valley, Nepal**

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The major earthquake (magnitude 7.8) occurred on April 25, 2015 in Nepal. An estimated 8670 fatalities and 2.8 million people were in immediate need of humanitarian assistance according to the government of Nepal. There were several aftershocks throughout the end of April followed by a major earthquake of 6.8 magnitude on May 12, 2015. Due to inadequate, interrupted, or intermittent treatment, public health authorities in Nepal have repeatedly associated waterborne disease outbreaks with unsafe drinking water. Nepal is also one of the poorest countries in the world and people can't afford water purification technologies such as the point-of-use (POU) filters which are effective on removing microbial pathogens. It is paramount that the earthquake victims have access to clean water and proper sanitation to prevent a major waterborne disease outbreak. At present, their major drinking water source is tanker water, which source is unknown and might be contaminated. During this catastrophic event, we conducted a preliminary qualitative study of drinking water quality and analyze free residual chlorine (FRC), Ammonia and coliform bacteria. However, we need to conduct a comprehensive and quantitative study of the problem of enteric pathogens in drinking water in various quake hit regions of Nepal. The purpose of this study is to assess the impact of earthquake in drinking water quality and household sanitation in kathmandu, Nepal. The project will provide information to local regulatory agencies that can be used to develop guidance for protection of drinking water supplies post-quake in Nepal. This presentation will cover our results of seasonal variation of opportunistic pathogens/overall bacterial microbiome in different sources of water in the Kathmandu Valley, Nepal.

**POP 50 Somatic coliphages threshold to improve management of activated sludge wastewater treatment plant effluents in resource-limited regions**

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Waterborne transmitted enteric viruses are a principal cause of diarrheal disease in infants and young children in developing countries. The insufficient removal of pathogenic viruses by conventional wastewater treatments methods brings about a high potential risk for public health. In this study samples from the influent and effluent of five urban wastewater treatment plants with activated sludge treatment, located in the Great Metropolitan Area in San José, Costa Rica, were obtained during 2013. Water samples were sampled during the dry and the wet season, and in transition season periods, for a total of 120 samples analyzed. Somatic coliphages, fecal coliforms and four enteric viral pathogens (Hepatitis A virus, Rotavirus, Enterovirus, and Norovirus) were analyzed using APHA protocols (single layer agar and double layer agar for somatic coliphages, and electronegative filter concentration plus RNA extraction and final point PCR for the pathogenic viruses). A statistically significant relationship between the concentration of somatic coliphages and the presence/absence of any of the four enteric viral pathogens was found in the effluents. Logistic regression analysis and ROC curves were used to estimate the somatic coliphages concentration threshold at the effluent. An acceptable discrimination threshold concentration of 3000 PFU/mL somatic coliphages was determined for monitoring the treated effluent wastewater for viral pathogenic risk. The threshold shows specificity of 75% and a Positive Predictive Value of 71% and can be useful in order to diminish the risk of viral particles discharge in tropical wastewater treatment plants without tertiary and quaternary treatment.

**POP 51 Quantification of human enteropathogens in urban matrices at Rio de Janeiro by qPCR and TaqMan Array Card**

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The dissemination of waterborne enteropathogens contributes to the increase of diseases. Human adenovirus (HAdV) and human polyomavirus JC (JCPyV) are promising indicators of the human fecal contamination in the environment, due to its high host specificity, resistance to heat and the water treatments. The objective of this study was to evaluate the bacterial and viral indicators, besides other human enteropathogens in aquatic ecosystems of the Jacarepaguá Watershed (Rio de Janeiro, Brazil) using three methodological approaches. Forty-six samples were collected in two ecosystems of the region (Lagoon System and Untreated Sewage of Health Units and Athletes' Olympic Village), from 2016. Colilert® (IDEXX) method was used for bacteriological parameter, while for viral indicators the samples were concentrated by organic flocculation and quantified using TaqMan® qPCR System. Others enteropathogens were investigated by using TaqMan Array Card (TAC) and ViiA™ 7 Real-Time PCR System in 19 representative samples. All samples showed high concentrations of Escherichia coli, with averages of  $4.65 \times 10^6$  MPN/100mL. The HAdV and JCPyV indicators were detected in 80.4% (38/47) and 76.6% (36/47) of the samples, with mean values of  $4.3 \times 10^5$  genomic copies/liter (cg/L) and  $1.8 \times 10^7$  gc/L, respectively. PP7 was detected in 100% of samples. Different groups of viruses, bacteria, protozoa and nematodes were detected in samples analysed by TAC, with CT variation of 21 to 38. E. coli results showed that 99% of the samples studied were unsuitable for bathing and fishing activities. Regarding human viral indicators, similar concentrations were observed in the sewage and lagoon samples, evidencing high anthropogenic contamination in the ecosystems of this coastal urban by domestic sewage disposal. The impact of the dissemination of these pathogens on seawater along the coast of the tourist area of Barra da Tijuca will be evaluated in future studies.

## **POP SESSION THURSDAY**

**POP 52 What about *Giardia*? Exploring monitoring and regulatory frameworks when a city's raw water source has more *Giardia* than *Cryptosporidium***

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In both Canada and the United States, guidance for determining treatment requirements for *Giardia* in surface waters is limited to a basic 3-log (99.9%) removal/inactivation requirement. This would often not be a problem because ensuring adequate treatment for *Cryptosporidium* oocysts will likely ensure adequate treatment for similar quantities of *Giardia* cysts (which are less resistant to chemical disinfection). However, this begs the question of how to determine if 3-log treatment is sufficient for *Giardia* and what to do if this is not the case, particularly in instances where *Giardia* cyst concentrations greatly exceed *Cryptosporidium* oocyst concentrations.

The City of Calgary in Alberta, Canada has such a source in which *Giardia* concentrations have been about ten-fold greater than *Cryptosporidium* concentrations on average over more than a decade of regular raw water monitoring. To date, requirements developed by the provincial regulator have ensured sufficient treatment for *Giardia*, but it may be appropriate to transition toward a more contemporary and health-based approach. Many provinces implement Health Canada's guidelines for

enteric protozoa in drinking water. Those guidelines provide a quantitative microbial risk assessment model as a tool to aid understanding of risks to water systems, but do not provide specific guidance on monitoring or determining if additional treatment (beyond 3-log) is needed or how much.

Calgary's leadership and lengthy experience in assessment of protozoan contamination of its source waters and implementation of a reporting and compliance framework could be helpful for community water systems in North America and beyond where 3-log treatment for enteric protozoa may be insufficient. Monitoring practices, data management, reporting and notification requirements, operational action levels, defining compliance, and risk communication for plant operators and decision makers are discussed.

#### **POP 53      Derivation of the equivalent qPCR value for *Escherichia coli* to existing culture-based water quality standards for monitoring beaches in Michigan**

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A quantitative polymerase chain reaction (qPCR) method (Draft Method C) has been developed for *Escherichia coli* (*E. coli*) by the United States Environmental Protection Agency (USEPA). However, a beach notification threshold value is needed for this method and has not been determined. To implement Draft Method C for monitoring beaches in Michigan, the Department of Environmental Quality (DEQ) led a 3-year effort with fourteen labs to share 6,669 water samples from 108 beaches with the USEPA National Exposure Research Laboratory (NERL). Selected beaches were representative of both the upper and lower peninsulas of Michigan with urban and rural locations on inland lakes and the Great Lakes. Samples were analyzed by local labs using a state-approved and USEPA-approved culture method (Colilert) and by both NERL and the local labs using Draft Method C. Linear relationships between culture and Draft Method C results were determined. Final analysis was based on results from a combined total of 2,092 samples from 39 beaches with at least one exceedance of the Michigan water quality standard by culture, at least 10 paired samples with quantitative estimates for both methods, and a minimum R<sup>2</sup> of 0.6 between the methods per beach. From this analysis, a Draft Method C qPCR value of 1.863 log<sub>10</sub> copies per reaction was determined to be equivalent to the Michigan culture standard of 300 *E. coli* per 100 milliliters (ml). The new beach notification value for Draft Method C will be implemented for monitoring Michigan beaches and will improve the protection of public health by providing results in hours instead of the next day.

**POP 54 How do future changes in climate, population and investments in wastewater management affect the microbial drinking water safety requirements of large river catchments?**

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In this paper we assessed how future changes and investments in wastewater management along the large Danube River affect microbial pathogen concentrations and the required treatment to achieve a health-based target. The projected changes for other European regions were further considered. An integrative approach was used based on source-targeted characterization by faecal indicator bacteria, genetic MST markers, reference pathogens and modelling scenarios. The computational tool QMRAcatch was used, which integrates quantitative microbial risk assessment (QMRA) with hydrological process-based microbial water quality modelling. The projected increases in river low flows, combined sewer overflows (CSOs) and human population were shown to have only small effects on the required pathogen reductions for the studied site until 2050. According to previous climate studies, future projections for Europe vary depending on region and projection method. A sensitivity analyses considering the full range of projected changes of mean river flows (+/- 40%), increased CSO frequencies (up to +400%), and urban population growth (up to +90%) demonstrated effects more clearly. Disinfection of wastewater effluent alone could mitigate the reductions of enterovirus, norovirus, *Cryptosporidium* and Giardia needed during drinking water treatment by only 1.5 log<sub>10</sub> (95th percentiles) at maximum. The full effect of wastewater disinfection (up to 4 log<sub>10</sub>) could only be reached, if simultaneous provisions to prevent CSOs were taken into consideration. The results of this study imply that catchment protection measures are fully effective only, if targeted at both, continuous and sporadic events of faecal pollution discharges. The study has important implications for health-based drinking water safety planning along rivers. This work was supported by the LE07-13 project Groundwater Resource Systems Vienna in cooperation with Vienna Waterworks, the WWTF project ESR17-70 and the NFB project SC15-016. This is a joint investigation of the Interuniversity Cooperation Centre for Water & Health ([www.waterandhealth.at](http://www.waterandhealth.at)).

**POP 55 How can rapid online monitoring of *E. coli* improve recreational water quality management? Insights from Canada and New-Zealand**

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Microbial pollution of bathing waters is assessed worldwide using culture-based enumeration of fecal indicator bacteria (FIB) as their presence has been quantitatively associated with gastrointestinal illness risk in swimmers. However, infrequent sampling (a couple of samples per season to weekly or daily sampling) and typical >24h sample-to-result times prevent from time-efficient and safe management of recreational sites. In this study, we assessed how rapid online measurement of β-D-glucuronidase (GLUC) activity (as surrogate of *E. coli*) could improve beach water monitoring and management.

Four sites used for water-based recreational activities in Canada (3) and New-Zealand (1) were sampled to compare culturable *E. coli* enumeration with GLUC activity measurements using linear regression analysis to best fit enzyme-based threshold values to the respective national regulatory *E. coli* beach action values (BAVs). For two beaches, online monitoring of GLUC activity was further conducted at bi-hourly frequency to unravel the pollution dynamics at high temporal resolution during dry and wet weather.

Using a total of 84 to 281 grab samples, GLUC activity-based threshold values between 3.1 and 5.4 mMFU.100 mL<sup>-1</sup> were found to best fit the respective regulatory *E. coli* BAVs of 200 and 260 CFU.100 mL<sup>-1</sup> for Québec and New-Zealand. Near real-time online monitoring of GLUC activity enabled to characterize the timing, amplitude and frequency of pollution events at both beaches, thereby providing a wealth of information for improved decision-making and public health protection.

Based on the established enzymatic threshold values, we show that the time of beach closure can significantly be reduced compared to current monitoring practices while maintaining a safe access to the users. Considering the short-term (up to hourly) variations in microbial contaminants, especially in urban waters, GLUC activity-based BAVs in parallel to high frequency online monitoring could significantly improve beach management through same-day decision-making as opposed to infrequent culture-based monitoring.

#### **POP 56      Estimating the burden of waterborne disease acquired through recreational exposure to CSO-impacted waters in Philadelphia: a Quantitative Microbial Risk Assessment (QMRA)**

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**Background:** Combined sewer overflows (CSOs) are known contributors of human fecal pollution in urban waterways. Recreational activities including swimming, fishing, and wading in these waterways have been linked to waterborne disease. Quantitative microbial risk assessment (QMRA) is a cost-effective method for quantifying disease risk due to environmental exposure to pathogens. This study uses QMRA to estimate the number of illnesses expected due to recreational exposure to CSO-impacted waters in Philadelphia.

**Methods:** Inputs to the QMRA model include the number of individuals exposed, number of exposure events per person, and dosage of pathogen ingested per exposure. The number of individuals exposed and exposure events per person are determined using observational data collected summer 2016-2019 at three sites in Philadelphia. The dosage of pathogens ingested per exposure are from literature on the volume of water (ml/minute) ingested during recreational activities and the average duration of recreational activities (minutes/day). The expected concentration of enteric pathogens (*Cryptosporidium*, norovirus, *Giardia*, *Escherichia coli* O157:H7, and *Salmonella*) in the water bodies will be inferred from indicator organism (human *Bacteroides*) concentrations in samples collected summer 2016-2019 at the same sites (pathogens/ml). Data on the probability of infection per dosage ingested and the probability of illness per infection will also come from the literature. These inputs are fit to probability distributions using maximum likelihood estimation and the potential number of disease cases are determined using Monte Carlo methods.

**Results:** Following a final round of data collection in summer 2019, final model results will include an estimated number of disease cases per 1,000 recreators per day per activity at each CSO-impacted site.

**Conclusions:** Findings may advise prevention efforts to reduce the risk of waterborne disease in Philadelphia and other urban areas.

**POP 57      The MERA Investigation: A transdisciplinary approach to tropical beach management**

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The MERA Investigation integrates conventional water quality monitoring with advanced microbiology testing, epidemiology surveys, and ethnographic research to identify behaviors that put tropical beachgoers at risk of illness. During the past year, MERA investigators executed simultaneous water quality, epidemiological, and ethnographic studies involving over 5,000 beachgoers at a popular beach along the Pacific coast of Costa Rica. During 26 weekends, 156 river and ocean samples were analyzed for the following: human-associated *Bacteroides* HF183, fecal indicator bacteria (fecal coliforms, *Enterococcus* spp., and *Clostridium perfringens*), fecal viral indicators (male-specific and somatic coliphage and sewage-associated pepper mild mottle virus), and reference pathogens (*Salmonella*, *Cryptosporidium*, *Giardia*, human norovirus genotype I, and human adenovirus). Water samples were most frequently positive for *Salmonella* and *Giardia*; bacterial indicators often exceeded U.S. EPA and Costa Rican water quality limits for primary contact and HF183 was frequently quantified at concentrations associated with risks greater than the U.S. EPA health benchmark. The team initiated epidemiology surveys with over 5,000 beachgoers and more than 1,000 beachgoers completed the follow-up survey. Thirty-three percent of those surveyed reported illness within two weeks of visiting the beach and over 25% of those individuals reported illness that interrupted daily activities. Simultaneously, ethnographic assessments of beachgoer behavior were studied through more than 500 behavioral observations and semi-structured interviews with more than 80 stakeholders, including health practitioners, national and international beachgoers, and local tourism employees. Male beachgoers (both adolescents and men) were most likely to submerge their heads in coastal waters while swimming and playing in the waves, while women were more likely to wade and avoid getting their heads wet. Data from water quality, epidemiological, and ethnographic studies will inform exploratory quantitative microbial risk assessment (QMRA) to understand the risk of illness to beachgoers and identify management recommendations to promote safe beach recreation.

**POP 58      Measuring the relationships between rainfall, combined sewer overflows, and human fecal contamination along surface water bodies in Philadelphia, PA**

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**Background:** Many urban areas across the United States and globally have combined sewer systems in which sewage and stormwater are collected and managed together. During rain events, these systems can overflow, discharging human fecal contamination into local waterways. Recreational activities along these waterways may present a significant health risk. For this reason, this study will measure the impact of rainfall and combined sewer overflows (CSOs) on human fecal contamination along creeks and rivers in Philadelphia.

**Methods:** In summer 2017-2018, 28 samples were collected at three sites along creeks and rivers in Philadelphia (May-September during which recreational activities are more prevalent) and an additional 24 samples (8/site) will be collected in summer 2019. 100mL grab samples will be collected and analyzed using qPCR for human *Bacteroides* (HF183 genetic marker) following the latest United States Environmental Protection Agency protocol. Data on rainfall (mm per 15 minutes) and CSOs (hours of overflow per day) for up to 14 days prior to sample collection will be provided by the Philadelphia Water Department. Data will be analyzed using Spearman Rank correlations and using distributed lagged models in R version 3.5.2 to determine which daily lags (up to 14 days prior) of

rainfall and CSOs correlate with increased levels of human *Bacteroides*.

**Results:** Preliminary data analysis showed that human *Bacteroides* concentrations correlated with rainfall and CSOs during the day of sampling and up to 12 hours prior, suggesting that at these sites, CSOs and rainfall have an immediate rather than a lagged impact on surface water quality and subsequent disease risk to recreators.

**Conclusions:** Findings will provide insight on the persistence of HF183 in surface waters as well as inform recommendations as to when the risk of recreational waterborne disease may be the greatest following CSO events.

## **POP 59      Antibiotic Resistant Pathogens and Genes in U.S. Recycled Water**

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Bacterial resistance to antimicrobials and antibiotics is an urgent threat to public health worldwide. The Organization for Economic Co-operation and Development recently predicted a death toll of 2.4 million from antibiotic resistant bacteria in North America, Europe and Australia between 2015 and 2050. Studies of antimicrobial resistance in hospitals abound, but less is known about resistant bacteria in community settings. Wastewater that has been recycled for potable or non-potable use may constitute an exposure route for humans that is particularly troubling due to the presence of antimicrobial compounds in wastewater, which may select for antibiotic-resistant pathogens. In this ongoing study, wastewater treatment plants (WWTPs) producing reclaimed water in Florida and Virginia (USA), were sampled at various treatment stages and in the storage and distribution systems. Representative ESKAPE pathogens (*Enterococcus*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter*) were cultured on media with and without antibiotics. PCR confirmation of phenotypic resistance among isolates obtained to date include ceftazidime-resistant *P. aeruginosa* in influent and after UV treatment, vancomycin-resistant *Enterococcus* in influent, cefotaxime-*E. coli* in primary effluent, activated sludge, and post-secondary/ozonation. Non-target screening using UPLC MS/MS identified around 100 personal care products, including antibiotics, in WWTP samples at pre-disinfection stages. This number decreased to 40-50 post-chlorination and in the distribution system. Metagenomic DNA sequencing analysis is underway and will provide insight into how the broader resistome, including antibiotic resistance genes, metal resistance genes, and mobile genetic elements, shifts during production of water for potable and non-potable reuse. The role of disinfectants in selecting sensitive and resistant bacteria is of special interest and will be investigated targeting *Enterococcus faecium* and *P. aeruginosa* as model strains, along with whole genome sequencing of select multi-resistant isolates. This project will provide new insights into preventing dissemination of antibiotic-resistant pathogens and their genes via water reuse.

## **POP 60      Environmental Antibiotic Resistance Monitoring: Linking Environmental and Clinical Surveillance**

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Antibiotic resistance in pathogenic bacteria is a major threat for human health. Evidence suggests that the environment is a reservoir for the evolution and dissemination of antibiotic resistant bacteria (ARB) and contributes to the prevalence of ARB in urban and clinical settings. Standardized monitoring for ARB in clinical settings has increased in recent decades, yet there is no standard method for monitoring ARB in the environment. The ideal method should detect and quantify high

consequence ARB and produce isolates that could be linked to clinically relevant strains. Here, we evaluate a method for monitoring environmental ARB in surface water by incorporating 1 µg/ml cefotaxime into a standard water quality method for quantification of *E. coli*. Resistant isolates are recovered, confirmed phenotypically, and analyzed by whole genome sequencing. The sequence data can be compared to national clinical databases, such as NARMS and PulseNet. This method has effectively differentiated between susceptible and resistant strains in pure culture and spiked surface water samples. Since September 2018, CDC has partnered with the Chattahoochee Riverkeeper to collect surface water samples from sewage-impacted areas. Water samples were collected at ten sites biweekly. To date, 75% of surface water samples had  $\geq 1$  MPN cefotaxime-resistant *E. coli*/100 ml, with concentrations increasing to 248 MPN/100 ml after rainfall. The proportion of cefotaxime-resistant *E. coli* is not stable, ranging from 0 to 9.4% of total *E. coli*. All recovered isolates have carried *blaCTX-M* or *blaCMY*, which are clinically relevant extended-spectrum  $\beta$ -lactamase genes, and 94% of isolates are multidrug resistant. The majority (78%) of characterized isolates are whole genome MLST types associated with extraintestinal or uropathogenic *E. coli*. By linking environmental isolates to clinical surveillance, we will be able to more accurately assess the health risk associated with environmental AR exposures.

## **POP 61      Source tracking of antimicrobial resistance in emerging countries**

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### **Introduction**

The health impact of antimicrobial resistance (AMR) in rivers, including resistant bacteria (ARB) and genes (ARG), is greatest in low-to-middle income countries (LMICs), where antibiotics are used less prudently and waste management is inconsistent. However, predicting AMR exposures is difficult because monitoring programmes rarely include ARB or ARG indicators. As such, an urgent need exists to characterise AMR in LMIC rivers, both to define current conditions, but also to develop models for predicting conditions with limited data.

### **Objectives**

1. Collect ARB and ARG data for a Malaysian river catchment 2. Develop and parameterise a hydrologic-AMR tracking model to health exposure studies

### **Methods**

Water samples were collected at 8 locations during 3 seasonal campaigns ( $n = 39$ ) across a rural-to-urban Malaysian river catchment (300 km<sup>2</sup>). Analyses included standard water quality parameters, antibiotics concentrations, culturable resistant bacteria (ESBL and carbapenem), resistomes and microbiomes (high throughput qPCR and sequencing). A model using HSPF BASINS was developed to simulate AMR transport in the catchment.

## **Results**

Monitoring showed culturable ESBL and carbapenem AR coliform counts increased by two and one log<sub>10</sub>, respectively, as one moved rural to urban parts of the catchment, although side tributaries caused spiked increases at intermediate locations. Highest ARB counts were  $2 \times 10^4$  and  $5 \times 10^2$  CFU/mL, respectively. Accounting for seasonal river flows, ESBL and carbapenem ARB transport loads were greater, by three and two log<sub>10</sub>, respectively. Early ARG data suggest a similar trend. Statistical comparisons with national sampling data show river dissolved oxygen (DO) level is a good surrogate for predicting AMR exposure. As such, DO was used to model AMR transport in HSPF, which being tested against new AMR data.

## **Conclusion**

Combining new AMR monitoring with existing national datasets can predict AMR exposures in LMIC river catchments.

## **POP 62     Extracellular antibiotic resistance gene decay by free chlorine treatment**

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Natural transformation is a major mechanism of antibiotic resistance acquisition where bacteria uptake and integrate the antibiotic resistance genes (ARG) located in extracellular DNA (eDNA). eDNA is present in aquatic environments and many bacterial strains have shown to be naturally competent to uptake eDNA. However, studies on the functionality of ARGs available in eDNA exposed to disinfectants in transforming environmental bacteria to become antibiotic resistant are limited.

In this study, we evaluated the effect of free chlorine disinfection on the functionality of an ARG in eDNA. Competent cells of *Escherichia coli* were used as the model of extracellular ARG recipient, and pUC19 (2686 bp, ampicillin resistant plasmid) was used as model eDNA. pUC19 was treated with free chlorine (initial concentration = 1ppm) for 0, 1, 2, 4 and 10 minutes. After that, solution was neutralized using sodium thiosulphate. Transformation of pUC19 in the free chlorine treated samples to *E. coli* was assisted with heat shock treatment. After overnight cultivation at 37°C in agar plates containing ampicillin, transformation efficiency was between  $6.0 \times 10^{-4}$  and  $5.7 \times 10^{-6}$ . Extracellular ARG decay constant (*k*) due to free chlorine treatment was calculated as 0.18 using Chick-Watson model (*P* value = 0.00146). A CT value of 6 ppmmin caused 50-fold reduction in the viable pUC19 plasmid.

Currently, information on the proliferation of ARB in aquatic environments by natural transformation, and the level of ARG reduction necessary during wastewater treatment to minimize the acquisition of ARG by environmental bacteria are limited. This study provides insights in terms of the extent of contribution by ARGs located in eDNA found in aquatic environments on the human health risks caused by ARB.

**POP 63 Sequential bio filtration of WWTP effluent for elimination of antibiotic resistant bacteria, viruses and indicator bacteria**

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In regions with limited water resources, groundwater recharge through potable water reuse is a conceivable alternative to improve water supply. However, a wide range of pathogenic bacteria and viruses as well as trace organic chemicals can be present in the final effluent of wastewater treatment plants. Biologically active filtration can provide low-cost water treatment. As bacteria in the filter preferably remove many organic compounds under oxic conditions, a sequential approach combining two infiltration systems with intermediate aeration (sequential managed aquifer recharge technology - SMART) can enhance the removal efficiency.

In this study, intermediate aeration with hydrogen peroxide and ozone was tested in two parallel silica sand column systems fed with effluent of a municipal wastewater treatment plant. The removal of antibiotic resistant bacteria (ARB) and resistance genes (ARG), viruses (coliphages and adenovirus) and indicator bacteria was examined with original WWTP effluent and after spiking.

In the initial phase, major elimination occurred in the first column (oxic) of each column set. The average reduction of *E. coli*, enterococci, somatic and f-specific coliphages and ARB was between 1.2 and 1.8 log. ARGs were not removed. Additional aeration in the second column enhanced removal rates of coliphages and bacteria (average 3.5-log reduction). The reduction of ARGs was increased to 1.4 log.

Presented results show that extended oxic conditions can improve removal efficiency. The sequential treatment provides a raw water quality that demands less complex treatment for subsequent drinking water generation.

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**POP 64 Genomic characteristics of carbapenemase-producing *Enterobacteriaceae* in hospital and municipal wastewater**

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The emergence of *Enterobacteriaceae* harboring carbapenemase-encoding genes is of great concern. In the present study, using whole-genome sequencing we characterized carbapenemase-producing *Enterobacteriaceae* (CPE) isolated from Japanese and Taiwanese wastewater. In total, 45 isolates were successfully sequenced and subjected to the genomic analyses. At least one carbapenemase-encoding gene was detected in 38 isolates. The remaining seven isolates did not carry carbapenemase-encoding genes, but they carried class C β-lactamases. Decreased outer membrane protein expression in combination with the overexpression of these class C β-lactamases seemed to be the main reason for the carbapenem resistance in these isolates. Among the carbapenemase-encoding genes, *blaGES* (*blaGES-5*, *blaGES-6*, and *blaGES-24*) genes were the most common and detected in 29 isolates. These genes were situated within class 1 integrons. In 1441 (cassette array, *blaGES-5-blaOXA-17*) was prevalent among *Escherichia coli* (n = 6), *Klebsiella* spp. (n = 11), and *Enterobacter cloacae* complex (n = 1) isolates from Taiwanese municipal and hospital wastewater. In 1442 (cassette array, *blaGES-6-aacA4-blaOXA-17*) was detected in *Klebsiella* isolates (n = 4) from Taiwanese municipal

and hospital wastewater. In1439 (cassette array, *blaGES-24-aacA4*) was found in one *Klebsiella variicola* isolate from Japanese municipal wastewater. In1440 (cassette array, *blaGES-5-aacA31-catB8-aadA5*) was detected in one *Klebsiella pneumoniae* isolate from Japanese municipal wastewater. Other carbapenemase-encoding genes (*blaVIM-1*, *blaNDM-5*, *blaIMP-8*, *blaIMP-19*, and *blaKPC-2*) were also found among one to three isolates. These results indicate contamination of wastewater by CPE harboring various carbapenemase-encoding genes, highlighting the need to monitor for antibiotic resistance in wastewater.

## **POP 65      Antibiotic resistant bacteria in Bavarian bathing waters**

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The quality of European bathing waters is monitored by detection of faecal indicators (*Escherichia coli* and intestinal enterococci) according to the EU Bathing Water Directive. No other microbial parameters are routinely examined (apart from cyanobacteria in some cases) and faecal indicators are not tested for antibiotic resistance. Transmission pathways of antibioticmicrobialresistance are not well understood and especially the role of the environment is not clear. There is little information about antibiotic resistant bacteria (ARB) in German bathing waters. Therefore it is not known, if humans encounter relevant concentrations of ARB by bathing in natural waters.

In this study 20 Bavarian bathing sites were analysed for the occurrence of clinically relevant antibiotic resistant bacteria in 2017 and 2018. 117 samples were taken – per bathing site one sample before and four to five samples during the bathing season. Water samples were concentrated by membrane-filtration and antibiotic resistant bacteria were isolated by placing the filters on different selective media. The isolates were identified by MALDI-TOF-MS and analysed for antimicrobial susceptibility by BD Phoenix™ and agar diffusion tests. The results were interpreted according to EUCAST guidelines.

Vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus* were not detected in any sample. However, at ten out of 20 bathing sites multiresistant Gram-negative bacteria were found (26 isolates of the following species: *E. coli*, *Serratia* spp., *Pseudomonas* spp., *Acinetobacter* spp.). Concentrations of multiresistant bacteria were low (median: 10 cfu/100 ml).

Detecting multiresistant bacteria in natural bathing waters was not a surprise since antibiotics are widely used in human and veterinary medicine. In our opinion bathing at designated Bavarian bathing sites poses a very low risk to human health. Only persons with special risk factors (e.g. large open wounds, serious immunodeficiency) should abstain on principle from bathing in natural waters – regardless of whether multiresistant bacteria were detected or not.

## **POP 66      Antibiotic-resistant bacteria in Danube river – is there a risk for bank filtration?**

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Antimicrobial resistance (AMR) is one of the leading public health concerns. The emergence of antibiotic resistant bacteria in surface waters poses a potential threat for drinking water production.

The current research in the framework of the consortial project “Clean water” aims to investigate the effect of source water quality and hydrometeorological factors on the efficiency of the bank filtration and the public health risk associated with the produced drinking water. The presence of ESBL-producing Enterobacteriaceae, vancomycin-resistant *Enterococcus* sp. and ciprofloxacin-resistant bacteria was investigated by-weekly in the Danube (upstream and downstream from Budapest), and from the water supply system.

Culture methods were used to retrieve AMR bacteria (Colilert broth supplemented with 10 mg/l ceftriaxone, Slanetz-Bartley agar with 8 mg/l vancomycin, and heterotrophic plate count agar (HPC) and blood agar with 1 mg/l ciprofloxacin). The same media without antibiotics were used to obtain background counts. Isolates were identified by MALDI-TOF MS.

The ciprofloxacin-resistant bacteria were detected in highest numbers among the targeted groups (up to 10 %). *Aeromonas*, *Acinetobacter* és *Escherichia* were the most often identified genera on ciprofloxacin supplemented blood agar. The rate of ESBL-producers within the detected Enterobacteriaceae remained below 1%, but 90% of the surface water samples were positive. Vancomycin-resistant enterococci were rarely isolated from the samples.

Enterobacteriaceae and enterococci were not detected from the bank filtered wells or the water supply system. Some ciprofloxacin-resistant bacteria were isolated from treated water as well; mainly belonging to the *Brevundimonas* genus.

Results confirmed that clinically relevant AMR bacteria are present in the Danube near Budapest. These bacteria are eliminated in normal circumstances during bank filtration. However, the efficiency under extreme high and low water flow required further investigation.

The research was funded by the Hungarian National Research, Development and Innovation Office (Grant No.: 2018-1.2.1-NKP-2018-00011).

#### **POP 67 Decay of the Antibiotic Resistance Genes *tetA* and *sul2* in the Water Environment: Opportunities for Sunlight Inactivation and Environmental Transmission**

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New York University

Antibiotic resistant bacteria (ARB) are a serious global health threat. Since ARB are excreted in the feces of infected individuals, their presence in human and animal waste presents a challenge in the design of water and wastewater treatment processes. While ARB can be inactivated by engineered disinfectants used at wastewater treatment plants, antibiotic resistance genes (ARG), the genes that code for resistance, often persist through the treatment process and end up in the environment. ARG can continue to pose a serious danger to public health given that bacteria containing ARG can convey resistance to surrounding bacteria through horizontal gene transfer mechanisms, such as transformation. Horizontal gene transfer contributes to the propagation of resistance and the creation of multidrug resistance. Additionally, many regions of the world are not served by wastewater treatment plants and ARB and ARG may be discharged into the environment untreated. Therefore, understanding the fate of ARB and ARG in the environment is essential in order to design control measures, predict health risks, inform ARG surveillance activities, and prioritize policy interventions. The objective of this study was to quantify and model the sunlight decay of ARG in order to better inform the development of proper environmental monitoring and control systems. Data from sunlight photolysis experiments conducted on the resistance genes *tetA* and *sul2* from tetracycline and sulfonamide resistant *E. coli* will be presented in the context of environmental decay of other microbial contaminants of concern. ARG were quantified using (1) qPCR protocols used in environmental surveillance and (2) long amplicon (LA)-qPCR methods previously described in the literature. Preliminary results from experiments conducted in photosensitizer free water indicate that while sunlight inactivation occurs, LA-qPCR is more sensitive at detecting DNA damage than assays more commonly used in environmental surveillance.

## **POP 68      Culture-Independent Detection of Antibiotic Resistance Genes and Bacterial Pathogens with DNA Microarrays**

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Detection of antibiotic resistance in a fast and reliable assay is essential for a quick risk assessment. Culture-independent methods are advantageous as they skip the time intensive culture step and do not discriminate against non-cultivable bacteria. Multidrug resistant bacteria are of great interest due to a large variety of problems caused by them in human and animals alike. Extended spectrum beta-lactamase (ESBL) producing bacteria are one example thereof, which convey resistance against several  $\beta$ -lactam antibiotics. An important gene class coding for ESBLs are **bla**CTX-M genes which were chosen as a target for detection of antibiotic resistant bacteria via DNA-based methods. Here, a DNA microarray based on heterogeneous asymmetric recombinase polymerase amplification (haRPA, Kunze et al., 2015) was adapted to detect all *bla*CTX-M cluster 1 genes in different bacterial species (*Klebsiella pneumoniae* and *Escherichia coli*) and the bacteria *K. pneumoniae* and *Pseudomonas aeruginosa*. The haRPA allows for sensitive and rapid quantification and qualification of specific DNA sequences in less than 1 h (after DNA extraction). The assay for *bla*CTX-M cluster 1 detection was compared to qPCR to evaluate its performance and we achieved comparable results. In future development of the assay, the combination of multiplex detection (several different gene sequences in one single measurement) and viability haRPA (distinction between living and dead bacteria using propidium monoazide treatment (Kober et al., 2018)) is a promising tool that will allow for faster screening of environmental samples for the presence of antibiotic resistance genes and pathogenic bacteria.

### References:

- Kober C, Niessner R, Seidel M, Biosens Bioelectron, 2018, 100, 49 – 55.  
Kunze A, Dilcher M, Abd El Wahed A, Hufert F, Niessner R, Seidel M, Anal Chem, 2015, 88 (1), 898 – 905.

## **POP 69      Impacts assessment of WWTPs effluent on antimicrobial resistance dissemination of receiving aquatic ecosystem in Japan by whole-genome sequencing approach**

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Wastewater treatment plants (WWTPs) are generally considered as hotspots for the spread of antibiotic resistance into aquatic environment due to biological treatment process can't effectively remove both antibiotic resistant bacteria (ARB) and the relevant genes. However, there is limited data on discussing the sources, presence, spread of ARB and significance of ARB from WWTPs effluent in aquatic receiving environments. The aim of this study was to investigate the presence and spread of antimicrobial resistance among *Escherichia coli* (*E. coli*) isolated from WWTPs effluent and Lake Biwa and to characterize *E. coli* isolates by microbial source tracking, multi-locus sequence typing, and analysis of *E. coli* phylogenotypes. A total of 93 *E. coli* isolates (51 from Lake Biwa and 42 from WWTPs effluent) were characterized through whole genome sequencing. As a result, we found that high prevalence of antibiotic resistant *E. coli* was detected in both WWTP effluent (21.4%) and Lake Biwa (35.3%) and the isolate which contained 11 different kinds of resistance gene also be found in WWTP effluent. Different distribution patterns of phylogenetic types and host sources were observed between isolates from effluent and Lake Biwa.

Moreover, 49 % of *E. coli* isolates from Lake Biwa were classified from chicken and cow, which were significantly higher than those from WWTPs effluent (17.9%). Those results suggest that livestock industry and wild animal can be considered as one of pollution sources as well. Based on our results we conclude that WWTPs can be seen as an important route for spreading antibiotic resistance, particularly multidrug resistance, into the environment, But WWTPs effluent is not the only source of ARB dissemination, other possible causes (e.g. livestock industry, waterfowl) have to be further considered.



# **POSTER PRESENTATIONS**

## POSTER SESSION MONDAY

### P 001 High-throughput 16S rRNA gene amplicon sequencing for the characterisation of the drinking water microbiome from an alpine karst spring

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Alpine karst aquifers are important groundwater resources for the provision of drinking water all around the world. Yet, still to date, very little knowledge is available on the composition and dynamics of the microbiome of drinking water resources. The aim of the present study was the characterization of the bacterial microbiome of spring water from an alpine limestone karst aquifer (LKAS2) and the study of its dynamics in response to changing hydrological conditions (base vs. event flow). The study was based on high-throughput 16S rRNA gene amplicon sequencing and a hydrology- and pollution microbiology data-guided study design and sample selection. Spanning more than 27 months, our analyses revealed a taxonomically highly stable bacterial community in the spring water of the studied alpine karst aquifer. Only the three yet uncultivated bacterial candidate phyla Parcubacteria (OD1), Gracilibacteria (GN02) and Doudnabacteria (SM2F11) together with *Proteobacteria* and *Bacteroidetes* contributed between 70.0 and 88.4 % of all sequence reads throughout the investigation period. The 300 most-abundant OTUs consistently contributed a fraction of 37.6 to 56.3 % of all sequence reads, supporting the previously proposed hypothesis of a high stability in the spring water microbiome. Yet, a clear response in the bacterial community could be observed during the studied high-discharge event caused by a summer storm event. Sequence reads affiliated to the class of *Flavobacteriia* clearly increased from a mean proportion of 2.3 % during baseflow to a maximum of 12.7 % during the early phase of the studied event, suggesting direct impacts from changing hydrological conditions on the bacterial community structure in the spring water. The variable populations within the bacterial class of *Flavobacteriia* might harbor potential as a valuable natural indicator of 'system disturbances' in karst aquifers. The work of this study was funded by the FTI-program of the province of Lower Austria.

### P 002 Chicago Area Waterway System Microbiome Research – Revealing Microbial Community Diversity

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The Chicago Area Waterway System (CAWS) is an urban river system located in the Midwestern region of the United States of America. The CAWS predominately receives the treated effluent from Metropolitan Water Reclamation District of Greater Chicago (MWRD). A seven-year CAWS microbiome research is being conducted in partnership with the Argonne National Laboratory with

sample collection from river water, including sediment and treated effluent discharged from two MWRD's plants during three years (2013–2015) prior to and two years (2016–2017) following disinfection by two different methods, UV and chlorination/dechlorination. The 16S rRNA amplicon sequencing (16S) was utilized to characterize the CAWS microbial communities.

Approximately 56 million paired-end reads were generated following the Earth Microbiome Project standard protocols in the Quantitative Insights into Microbial Ecology and DeBlur pipelines for Exact Sequence Variants picking. The CAWS sediments were the most diverse, followed by sewage, effluent, and CAWS water samples. Microbial composition was relatively stable during the sampling period from 2013 to 2015, but post-disinfection years (2016–17) showed reduction of known sewage/human fecal indicators. The comparison of fecal bacteria by traditional culture method and 16S demonstrated low abundance of *E. coli* sequences in the CAWS. Using the compositional analyses of the 16S data, we identified a significant post-disinfection reduction of known sewage and human fecal indicators such as *Acinetobacter*, *Cloacibacterium*, *Bifidobacterium*, and *Clostridiales* in both river water and sediment samples. There were significant differences between the two years i.e. 2016 and 2017 post disinfection. We observed a further reduction in sewage indicators such as bacterial taxa Lachnospiraceae, Paraprevotellaceae, Bacteroides, and Clostridiales in 2017 when compared to 2016. The tributaries feeding to river showed dominance of bacterial genera Polynucleobacter, Sediminibacterium, Acinetobacter, Arcobacter, Rhodobacter and Pseudomonas.

To date, this is the first longitudinal and spatial metagenomics study providing information on CAWS microbial community changes with the MWRD's environmental stewardship efforts to restore and maintain river quality.

#### **P 003      Next generation sequencing (NGS) for the analysis of antibiotic resistance genes in hospital wastewaters: a multicenter survey in Romania**

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#### **Objective**

Profiling of antibiotic resistant bacteria (ARB) and AR genes (ARG) in wastewaters from hospitals (hospital sewage and post-treatment effluent) as correlated with their identification in samples from patients admitted to Infectious Diseases Hospitals.

#### **Methods**

Hospital wastewater samples (different stages, influent, effluent, with/without chlorination) were collected using the National Guidelines. The hospital laboratory provided ESKAPE strains isolated during the week preceding wastewater sampling.

Wastewater filtration was performed and ARB were isolated, identified by MALDI-TOF and analyzed for their antibiotic susceptibility profiles. Strains with similar resistance phenotypes from hospital and environmental samples were selected for NGS analysis.

Eighty bacterial isolates were sequenced on a NGS platform (Miseq, Illumina) by using the paired-end shotgun strategy. The sequencing reads were mapped using Geneious Prime software. *De novo* assembly was performed when needed. Genotyping, resistance and virulence genetic markers identification was performed using several online tools.

#### **Results**

All ESKAPE strains were isolated from clinical sources and wastewater samples. Pan-resistant bacterial strains were found in some cases. For all strains, phenotyping and genotyping resistance testing gave concordant results. Both chromosomal and plasmidial ARG markers were found. The most prevalent chromosomal resistance genes were *fosA* and *blaSHV-1* for *Klebsiella pneumoniae*,

*VanA* and *msr(C)* for *Enterococcus faecium*, *blaOXA-66*, *sul1*, *armA*, *msr(E)* and *mph(E)* for *Acinetobacter baumannii*. Plasmid resistance genes were: *aac(3)-IIa*, *sul1* and *dfrA12* in *K. pneumoniae* and *ant(6)-IA* in *E. faecium*. Some of the MLST profiles observed in clinical strains were found in wastewater as well (prior to chlorination). Furthermore, some of the pre-chlorination MLST profiles were observed post-chlorination as well, albeit with a low density.

## Conclusion

NGS coupled with bioinformatic analysis are powerful tools to monitor the depletion in resistance genes originating from clinical settings and through the process of wastewater treatment. MLST analysis showed in some cases ARB continuity from hospital to post-processed water.

### P 004 Microbial community structure and diversity of activated sludge in wastewater treatment plants located in different climate zones of Australia

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The pathogen removal efficacy of wastewater treatment plants (WWTPs) using conventional activated sludge (AS) process depends upon design, operational parameters, and climatic conditions. The influence of diverse geographies and climatic zones on the microbial diversity and community structure of activated sludge process remains relatively unexplored. Deep sequencing and analysis of the 16S rRNA gene were performed to explore microbial diversity and community structure of five WWTPs represented diverse geographies, different climatic zones, operation parameters, and chemical conditions.

At the phylum level, Proteobacteria (44%), Bacteroidetes (33%) and Firmicutes (11%), predominated. The most commonly identified families were Pseudomonadaceae (15%), Saprospiraceae (15%), Chitinophagaceae (7%) Oxalobacteraceae (5%), Moraxellaceae (3%), Comamonadaceae (3%), Rhodocyclaceae (3%), Porphyromonadaceae (3%) and Clostridiaceae (3%). More than 12500 operational taxonomic units (OTUs), corresponding to 1400 unique genus-level identifications, were detected in six WWTPs with high diversity across plants. This supports the concept of a stable core microbiome in AS linked to WWTP functions. The dominant bacterial functions in different WWTPs were similar. The AS microbial communities were influenced by deterministic factors such as organic C content and temperature.

A total of 12 genera known to carry potentially pathogenic bacteria were detected in the AS samples with Clostridium more commonly detected in tropical and subtropical WWTPs. The functional profiles of bacterial communities including antibiotic resistance genes (ARGs) in the AS were then predicted using PICRUSt. Efflux pumps were found to be the most common antibiotic resistance mechanisms. This study advances the understanding of the diversity and biogeography of AS microbial communities, potential pathogenic microorganism, and ARGs in different WWTPs.

**P 005      Bacterial diversity and community structure in a Mediterranean stream affected by a wastewater treatment plant**

Miriam Pascual-Benito, Elisenda Ballesté, Anicet R. Blanch, Francisco Lucena and Cristina García-Aljaro

University of Barcelona

The metagenomic analysis of water along a river's course is a very useful tool to determine the impact of wastewater treatment effluents in the stream communities and the possible effects on the water quality.

In this study, 16S rRNA amplicon sequencing was used to assess the impact of the effluent of a wastewater treatment plant (WWTP) on the water quality of the *Riera de Cànores*, an intermittent Mediterranean river. Seven sampling campaigns were performed under different temperatures and stream flow conditions (winter and summer). Water samples were collected upstream (UP) of the WWTP, and 75 m (P75) and 1000 m (P1000) downstream of the WWTP. The effluent of the WWTP (EF) was also analysed. 1 L of each sample was prefiltered through a 3.0 µm pore size membrane, followed by 0.22 µm pore membrane, from which DNA was extracted. The V4 hypervariable region of the bacterial 16S rRNA gene was amplified using the Illumina MiSeq sequencing platform.

A total of 4,388,681 sequences were obtained deriving into 21,000 sequence variants (SV) which were further analysed and classified based on phylum, class, family and genus.

Highest alpha-diversities (Shannon) were observed for UP and P1000 samples. Furthermore, seasonal differences were observed in the alpha-diversity, being higher in winter with a high river flow and low temperature and river bioreactivity, therefore indicating a possible influence of the environmental parameters in the bacterial community structure.

Each sample presented different distribution and abundance of taxons ( $\beta$ -diversity). In general, the most abundant phyla in UP samples were Proteobacteria and Patescibacteria. The community structure of P75 resembled to that of EF samples, whereas UP and P1000 samples mostly clustered together, suggesting that the autochthonous river communities can be recovered from the impact of the WWTP effluent in 1 km.

**P 006      Role of Water Treatment, Nutrients, and Physicochemical Factors in Regulating Viral and Microbial Composition in an Aquarium by Metagenomics Approaches**

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We aimed to (i) characterize viral and bacterial communities of cold and warm marine and freshwater human-contact aquaria habitats and (ii) examine the role of water treatment components and environmental parameters in shaping viral and bacterial populations. A total of 75 physicochemical, bacterial, and viral water samples were collected from three aquarium systems: cold marine (Oceanarium), warm marine (Wild Reef), and freshwater (Sturgeon Touch), and their water treatment components: sand filter, foam fractionators, and UV and ozone water disinfection at Shedd Aquarium in Chicago. Physicochemical tests (#23) were run by the Shedd Aquarium water quality laboratory, bacterial water samples were analyzed for amplicon-sequencing (16S rRNA, V3-V4) by Illumina MiSeq™ and viral water samples were sequenced by Illumina HigSeq™ 4000. Bacterial, archaeal, and viral composition varied significantly by sampled habitats ( $p=0.001$ ) and only archaeal composition varied with water treatment components ( $p=0.031$ ). Bacteria, archaea, and viruses had the highest diversity in freshwater storage, warm marine, and sea lion sand filter, respectively. Seven physicochemical parameters, pH, Alkalinity, NO<sub>3</sub>, Salinity, PO<sub>4</sub>, Temperature, and N02-N, correlated

with bacterial and archaeal compositions ( $R=0.4$ ,  $p=0.001$ ) and explained high variation (9 to 22.0%,  $p=0.001$ ) in community compositions, while only temperature and pH correlated with viral composition ( $R=0.38$ ,  $p=0.001$ ) and explained variation in viral composition of 16.4% and 10%, respectively. Viral pathogen-like sequences were enriched in cold marine and freshwater habitats, and were not detected after UV disinfection. Profiling bacterial, archaeal, and viral compositions of aquatic built environment provides insights on the microbial stability of aquatic systems.

#### **P 007 Capture and phylogenetic characteristics of ultramicrobacteria in groundwater**

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Flowcytometric investigations of water samples together with high-throughput sequencing methods provide deep insights into microbial community characteristics among different types of groundwater. The high abundance of low nucleic acid (LNA) cells in water, especially groundwater that is associated with small cell size indicate strong occurrence of ultrasmall bacteria. Separation of bacteria by cell size using gradual filtration have shown clear differences in their corresponding community compositions, but filtration down to 0.2  $\mu\text{m}$  pore size, that is usually the pore size assumed to absorb all bacteria, may not always be sufficient. Flowcytometric investigations of two bank filtrates and one treated water sample in a distribution system have shown that after 0.2  $\mu\text{m}$  filtration up to 30% of total cells can still be determined in water samples, thus strongly contributing to overall bacterial compositions. The inclusion of a subsequent 0.1  $\mu\text{m}$  membrane filtration has shown no quantifiable cells in the filtrate, proving the entire capture of the bacterial community. As LNA cells are prone to ultrasmall cell sizes, flowcytometry can provide a good screening tool for detailed characterization of the ultramicrobiome in water. Microbial communities that were captured on 0.1  $\mu\text{m}$  filters after previous 0.2  $\mu\text{m}$  filtration showed to be as viable as the remaining community and predominantly consist of bacterial groups belonging to the superphylum Patescibacteria, mainly Parcubacteria. Interestingly, in absolute numbers Patescibacteria contribute almost equally to the communities captured on 0.2  $\mu\text{m}$  filters as compared to 0.1  $\mu\text{m}$ , suggesting physical conjunctions to other bacteria as a consequence of intra-community dependencies.

#### **P 008 The Impact of Pipe Material on the Diversity of Microbial Communities in Drinking Water Distribution Systems**

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Drinking water distribution systems (DS) house a myriad of organisms that proliferate in pipe biofilms. These biofilms can harbor, promote, and release human pathogens into the bulk water. The biofilm microbiome can be affected by various factors, including pipe material, disinfectant residual, temperature, and flow rate.

To fill an important gap regarding biofilm composition in full-scale DS, we connected annular reactors (AR: systems simulating shear stress on pipe walls) to a major US city water main. Each AR contained 20 coupons made of pipe material upon which biofilm could accumulate. We studied one set of reactors (3 ARs) with cast iron coupons and another set (3 ARs) with cement coupons. One cast iron

and one cement coupon were aseptically sampled weekly from each set ( $n=78$ ). High-throughput sequencing of the 16S rRNA gene (V3-V4 region) was used to characterize the microbial communities and analyze the impact of pipe material and water quality parameters on the composition and temporal variability of the biofilms.

Compared to cement samples, cast iron samples were associated with a higher level of both alpha-diversity (Kruskal-Wallis;  $p<0.001$ ) and beta-diversity (PERMANOVA;  $p=0.001$ ). The relative abundance of *Legionella* were 0.4% (cement) and 2% (cast iron). In contrast, *Mycobacterium* abundance was 40% and 23% in cement and cast iron pipes, respectively. Temporal variability in diversity was also observed by pipe material. While diversity (Shannon/Simpson indices) was variable over time in the cement samples, diversity metrics were stable in the cast iron samples. These results suggest that cement pipe has less microbial competition and may promote pathogen survival, whereas cast iron pipes are more microbially diverse. As United States (US) water utilities replace cast iron pipes with cement or plastic pipes, we need a better understanding of the implications of changing pipe material on the microbial safety of distributed water.

**P 009 Characterization of plastic-associated bacterial community in freshwater lake of Vácszentlászló, Hungary**

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Despite the great benefits of plastics in different aspects of the life, and due to the increase in plastic production and use, plastic wastes are becoming of major environmental concern. It is well known that inappropriate use and disposal also lead to accumulation of plastic litter in different aquatic environments. Microbial biofilm is able to develop on the surface of plastics (plastisphere) in aquatic environments with time.

In our study it was aimed to describe the bacterial community associated with plastics in freshwater. Thus, a total of nine self-designed plastic colonizers were submerged under the surface of water in Vácszentlászló lake located in central Hungary, for a period of three months.

Three plastic colonizers were cultivated every month and bacterial community associated with plastic samples then were analyzed as follows: a.) culturable bacteria were isolated from plastic surface and identified by 16S rRNA gene sequencing b.) bacterial community was studied using terminal restriction fragment length polymorphism (T-RFLP) and followed by amplicon sequencing c.) besides these molecular analysis of plastic colonizing community, surface water samples from the lake was also taken and analyzed by the same methods.

By the results of aerobic isolation, the following notable bacterial groups were identified: bacteria related to fish spoilage, like *Bacillus simplex*, and bacteria with possible biodegradation capability like *Pseudomonas antarctica*, published as hydrocarbon degrader. Some bacterial isolates are also known as responsible for threatening environmental safety like *Aeromonas bestiarum* which is known as a fish pathogen and *Shewanella putrefaciens* which may be an opportunistic human pathogen.

By the results of T-RFLPs, the plastic colonizing community shows notable differences comparing to water samples, and it was also verified by the results of amplicon sequencing.

**P 010 Application of Different Next Generation Sequencing Strategies to the Characterization of Sewage Virome**

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Next Generation Sequencing (NGS) emerged as a promising approach to characterize viruses present in complex samples. This work aimed to evaluate the performance of different NGS strategies (High Throughput Sequencing, Target Enrichment Sequencing and Amplicon Sequencing) for the characterization of potentially pathogenic viral strains present in raw urban sewage.

Viral particles from a composite raw sewage sample were concentrated by ultracentrifugation and nucleic acids extracted after DNase treatment. Amplicon sequencing was performed by deep sequencing of a nested (RT)-PCR product obtained after amplifying specific viruses: Human papillomaviruses (HPV), Human adenoviruses (HAdV) and Enteroviruses (EV). For the High Throughput and the Target Enrichment Sequencing, nucleic acids were processed following a sequence-independent single-primer amplification method. Two libraries were prepared simultaneously, one was directly sequenced and the other one was captured with specific viral probes (VirCapBSeq VERT, Roche) and sequenced with an Illumina platform.

Amplicon Sequencing specific for EV resulted in 45 different EV strains detected, including EVA71, involved in a brainstem encephalitis outbreak occurring simultaneously to the sampling period. This strain, together with other 6, were also detected by Target Enrichment Sequencing. Amplicon sequencing of HPV resulted in 41 types including 2 oncogenic types and for HAdV, among 8 different serotypes detected the more prevalent were HAdV40 and 41 which are important gastroenteritis agents.

High Throughput Sequencing showed 3 different human viral families (*Astroviridae*, *Caliciviridae* and *Anelloviridae*) to be present in sewage and HPV, EV and HAdV members were only detected after capture with specific viral probes by Target Enrichment Sequencing together with other 9 families containing human pathogens (*Astroviridae*, *Caliciviridae*, *Anelloviridae*, *Reoviridae*, *Parvoviridae*, *Picornaviridae*, *Hepeviridae*, *Polyomaviridae* and *Picornaviridae*).

Amplicon Sequencing proved to be useful for study of viral diversity of specific families while Target Enrichment showed to be the better choice to obtain a whole picture of the virome in sewage.

**P 011 Metagenomic analysis of virus, bacteria and protozoa in irrigation water**

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Agriculture irrigation is increasing the total annual water use in Europe. Reclaimed water is a good alternative to reduce the dependence of conventional irrigation sources, but may have a direct impact on the quality of irrigated fruits and vegetables. Different viruses (e.g., noroviruses and hepatitis A and E virus), bacteria (e.g., *Salmonella* spp. and pathogenic *Escherichia coli*) and protozoa (e.g., *Cryptosporidium parvum*, *Giardia intestinalis*) are well-known contributors to reported food-borne illnesses linked to contaminated fresh produce, however it is known that the number of food-borne infections is underestimated and the list of pathogens uncompleted.

Within the Metawater project (Water JPI), the viral, bacterial and protozoan populations were monitored over one year period in different irrigation water sources (reservoir water, groundwater and river water) by using a metagenomic approach. A total of 18 season-pooled samples were tested after the concentration of viruses, bacteria and protozoa using the Skimmed Milk Flocculation (SMF) method. Raw sewage was also analysed as it is a representative source of microbial pollution.

Most of the known viral species (>77%) derived from plants and bacteriophages. The viral diversity in the river water shifted over seasons, increasing bacteriophage reads during autumn and winter, when Noroviruses GII were also detected. Reservoir water presented high levels of eutrophication; *E. cloacae*, considered as opportunistic human pathogen and a faecal contamination indicator, and *N. australiensis* and *N. clarki* were reported in this type of water. Emerging human pathogens as HEV and *Naegleria fowleri* were detected in groundwater during the summer sampling. A wetland used as a sustainable system, to treat secondary effluents from urban WWTP and produce reclaimed water, seems to restore the natural microbial community as the virome and bacteriome resemble those present in freshwater samples (river and reservoir water).

#### **P 012 Microbial diversity in a Full-Scale Waste Water Treatment Plant in St. Bernard Parish**

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Clean water is one of the most important natural resources on the planet. Therefore, the detection of viable bacteria in wastewater treatment plants (WWTPs) is very important for public health, as WWTPs are a medium with a high potential for waterborne disease transmission (Li D et al., 2018). The St. Bernard WWTP is a traditional wastewater treatment plant, meaning it undergoes primary and secondary treatment. Through these treatments, effluent from the sedimentation tank is disinfected with chlorine where 99% of the bacteria will be killed before being discharged into the Mississippi (EPA 1998). 16s rRNA illumina sequencing provides insight into pathogenic bacteria throughout the treatment process and how it can affect humans. In this study, we investigated the bacterial communities and pathogenic bacteria in the St. Bernard Wastewater Treatment Plant that utilizes a conventional waste water treatment. Although this study revealed that most of the pathogens were removed throughout wastewater treatment, some pathogenic bacteria was observed in the effluent stage. Consistent with many other studies, the major phyla in wastewater samples were Proteobacteria, (with Gammaproteobacteria and Betaproteobacteria among the top two classes), Bacteroidetes, Planctomycetes and OD1. Dominant genera were often members of Proteobacteria and Bacteroidetes including several pathogens of public health concern, such as Arcobacter, Chryseobacterium, and Mycobacterium. This study exemplifies how analysis of WWTP can be effective in understanding the quality and performance of WWTP for environmental health purposes. The WWTP in St. Bernard Parish is doing a sufficient job in reducing most pathogens from the influent to effluent stage. However, the opportunistic pathogens found in the effluent stage should still be concerned.

#### **P 013 Impact of rotavirus vaccination at differing levels of piped water and sewerage access: an analysis of childhood clinic visits for diarrhea in Peru, 2005-2015**

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Since 2005, Peru has added an oral rotavirus vaccine to the national immunization schedule, and increased access to piped drinking water and sewerage. We examined whether these factors were associated with the rate of clinic visits for diarrhea in children under five. We used negative binomial regression to analyze the impact of rotavirus vaccination and piped water and sewerage access on

diarrhea rates in the 195 Peruvian provinces from 2005-2015, considering the interaction between these factors, and controlling for long-term and seasonal (El Niño) trends. We compared the “pre-vaccine” (2005-2009) and “post-vaccine” (2010-2015) eras. Annual percentages of households with access to piped water and sewerage were analyzed separately in quartiles.

Higher access to piped water was associated with significantly lower childhood diarrhea rates in the post-vaccine era only; higher sewerage access tended to be associated with less childhood diarrhea in the post-vaccine era, but not in the pre-vaccine era. We found no significant effect of rotavirus vaccination in the lowest quartile of piped water access or sewerage access. Controlling for long-term trend, compared to the pre-vaccine era, the diarrhea rate was lower in the post-vaccine era by 7% (95% CI: 2-12%), 13% (95% CI: 7-19%), and 15% (95% CI: 10-20%) in the 2nd, 3rd, and 4th quartiles of piped water access, respectively. In the post-vaccine era, the diarrhea rate was 9% lower in the 2nd and 3rd quartiles of sewerage access, and 13% (95% CI: 6-19%) lower in the highest quartile. Diarrhea rates were significantly higher (6%, 95% CI: 4-8%) during moderate/strong El Niño events.

Improved access to piped water and sewerage may operate synergistically with rotavirus vaccination to reduce childhood clinic visits for diarrhea in Peru. Our results suggest that implementation of rotavirus vaccination without adequate provision of water and sanitation may reduce the health impact of vaccination efforts.

#### **P 014 Identifying populations at higher risk of exposure to combined sewer overflow-impacted waters in Philadelphia**

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**Background:** Recreational activities such as swimming, fishing, and wading can increase exposure to waterborne pathogens. This is particularly relevant for populations that recreate in urban waterways where combined sewer overflows (CSOs) discharge human sewage into surface water. Several studies have documented recreational activities in CSO-impacted waterways, however none have focused on identifying areas where exposure is most likely. This study will use geographic information systems (GIS) to identify areas where recreational exposure to CSO-impacted water is most likely and compare population demographics for those residing higher and lower risk areas.

**Methods:** High risk populations will be defined as those living within a specified travel distance of a public park space that contains a waterbody impacted by CSOs in Philadelphia. Low risk populations will include residents of Philadelphia living outside of this travel distance. Network-based travel time buffers will be created using the most common travel method and mean travel time reported in a survey of individuals recreating in Philadelphia's creeks and rivers (data collection on-going). The network analysis tool in ArcMap version 10.4 will be used to identify higher risk areas using Philadelphia street network and travel time data. Logistic regression models will be created to identify census tract-level demographic correlates of residing in higher risk vs. lower risk areas.

**Results:** Adjusted odds ratios will identify independent correlates of living in areas where recreational exposure to CSO-impacted waters is more likely in order to identify groups that are more likely to recreate in these waterways.

**Conclusions:** Findings may be used for waterborne disease burden estimates and to identify vulnerable populations that are more likely to be impacted by CSOs in Philadelphia and other urban areas.

## P 016 Effect of personal hygiene on norovirus transmission within and among households

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### *Background*

Norovirus is a leading cause of acute gastroenteritis in the world. To prevent the spread of diseases via humans and the environmental surface, previous studies have examined the importance of personal hygiene (e.g. hand washing, sanitizer spray, etc.), mainly focusing on clinical settings. However, an important public health question has yet to be clearly answered; the personal hygiene in our daily life was effective enough or not?

### Objectives

Our study aimed to estimate the probability of transmitting norovirus infection at household settings by the level of hygiene awareness to empirically quantify its effectiveness.

### Materials and Methods

#### Data

Using an internet-based questionnaire, we conducted a retrospective household survey and collected the history of norovirus infection and scores of personal hygiene habits from 439 households from October 2018 to February 2019.

#### Probabilistic model

Our previous studies had estimated the transmissibility of norovirus among household members, formulating the chain of infections given by household members or outside of the household. Our present study further extended the chain binomial model (an expanded Reed-Frost model) to include the heterogeneous transmissibility by age, sex, and the level of hygiene awareness.

### Result

The probability of infection within a household was almost the same among different levels of hygiene awareness. On the other hand, the probability of infection from outside of households was estimated to be 0.412 and higher in households with lower-level awareness (0.202) by Bayesian inference.

### Conclusion

The estimates indicated that personal hygiene might not be enough to control the spread of infection once norovirus invades into a household, because of the huge number of emitted viruses via infecteds, but may prevent its entry from outside of the household. Low-dose exposures (i.e., contamination on hands) might be reduced by individuals' behavior, resulting in the enhanced protection as a community scale.

## P 017 Circulation of Hepatitis E virus genotype 3 between humans and water

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Hepatitis E virus (HEV) is the main cause of acute hepatitis worldwide. In France, genotype 3 is the most frequent genotype, transmitted mainly by animals but hydric transmission is suspected.

Our aim is to explore HEV circulation between humans and the environment and the influence of HEV variability on viral fitness, pathogeny and transmission.

HEV positive samples were detected by qRT-PCR, all of genotype 3: 10 % of sera and 39 % of stool samples coming from patients with acute hepatitis, 20 % of the entrance of a wastewater treatment plant (WWTP) samples, 7.5 % of wild boars samples, 60 % of pig slaughterhouse effluent samples and 7.5 % of pure water mussel samples. According to sequencing data from the overlapping region ORF2/ORF3, the viral variability and predicted antigenicity of the corresponding proteins were analysed.

Considering this region analysed by ultra-deep sequencing, a major variant was found in every sample, with minor variants in various proportions, except for the sequenced WWTP samples showing a mixture of different variants, with average genetic distance within each sample between 0.002 and 0.046 while between 0.057 and 0.084 in the WWTP samples. The H81C modification (ORF3) in the major variant of all wild boars was also observed in 25 % of the reads of one of the WWTP samples, the L87F modification (ORF3) was observed in two patients (one of them HEV chronically infected); associated with a modulation of predicted antigenicity for H81C.

HEV was observed to broadly circulate in humans and the environment. WWTP samples contain a higher diversity of variants than other samples and contain human and animal strains, in favour of a role of water in the circulation of genotype 3 HEV. Molecular signatures observed in some samples suggest HEV adaptability according to its host and possible impact on HEV fitness.

## P 018 Establishing the prevalence of *Legionella pneumophila* in various niches of chlorinated drinking water distribution systems

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Amplification of *Legionella pneumophila* (*Lp*) in large building water systems is well documented. Low levels of *Lspp* and *Lp* ranging from 3-5.4% have been reported in drinking water distribution systems (DS). Recently, DS disturbances resulting in massive iron release and loss of disinfectant have been identified as factors contributing to the Genessee-Flint Legionella outbreak. This raises questions about the relative contribution of water quality in the municipal DW to the promotion of *Lp* growth in downflow building water systems. There is a need to quantify the significance of *Lp* seeding from DS, and to identify at risk areas such as fire hydrants, dead ends and areas of low flow. An ongoing field study was initiated in 2018 to evaluate the occurrence of *Lp* (Legiolert), *E. coli*/total coliforms (Colilert), indicator organisms, water quality (Cl<sub>2</sub>, Fe & metals, turbidity, particles, pH, etc.) and microbial diversity in four drinking water distribution networks. Two approaches were taken: (1) monitoring at fire hydrants, sites with high levels of iron, no disinfectant residual and very high water age; and (2) concentrating large volumes (100L) at the plants outlets (100L) and at taps (20L) of several buildings collecting first draw and fully flushed samples. Positive results for *Lp* were observed

in fire hydrant samples (3/28) and in large volumes collected from the distribution system (2/19), including a positive sample from a treatment plant. TC positive results were observed in 3/28 fire hydrants, and 1/19 for large volumes. Residual chlorine was present for all positive samples (0.26-0.81 mg Cl<sub>2</sub>/L). Sampling is ongoing, together with typing of the *Lp* and TC strains and 16S/18S analysis. Results from this study are important to understand the role of the distribution system in seeding building water systems, and to identify the potential of residual maintenance to minimize *Lp* in DS.

## P 020 Economic impact of harmful algal blooms on human health: a systematic review

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**Background:** Harmful algal blooms(HABs) damage human activities and health. While there is wide literature on economic losses, little is known about the economic impact on human health.

**Objectives:** In this review, we systematically retrieved papers which presented health costs following exposure to HABs.

**Methods:** A systematic review was conducted up to January 2019 in databases such as ScienceDirect and PubMed, and 16 studies were selected. Health costs included health care and medication expenses, loss of income due to illness, cost of pain and suffering, and cost of death.

**Results:** Two categories of illness (digestive and respiratory) were considered for health costs. For digestive illness cost, we found \$86, \$1,015 and \$12,605, respectively for mild, moderate and severe cases. For respiratory illness, costs were \$86, \$1,235 and \$14,600, respectively for mild, moderate and severe cases. We used Quality-Adjusted Life Years (QALYs) to access the loss of well-being due to illness caused by HABs. We found that breathing difficulty causes the most loss of QALYs, especially in children, with a loss of between 0.16 - 0.771 per child. Having gastroenteritis could cause a loss of between 2.2 - 7.1 QALYs per thousand children.

**Discussion:** Unreported illness could be by far the most important bias which may occur in estimating health costs due to harmful algae exposure. There is an incapacity to measure all the costs following exposure to harmful algae. Chronic long-term effects are more difficult to highlight. Misleading symptoms of illness following exposure to HABs could cause bias in health costs estimations.

## P 022 Emerging opportunistic bacteria during storage of common carp (*Cyprinus carpio*)

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The quality and safety of freshwater fish are greatly affected by the microbial community, leading to a relatively short shelf life of fish products. Some special Hungarian lakes of sodic area are presumed to influence the microbial community of common carp (*Cyprinus carpio*), the key species of Hungarian fish production. Therefore, the aim of this work was to follow the microbial changes of stored carp fillet originating from this area, with a special focus on emerging opportunistic microorganisms.

Carp fillets were stored for 4 days on 2-4°C, 6-8°C with and without vacuum package. Fillets were periodically examined by standardized microbial and molecular methods. Community profiles of the stored fish samples were determined by T-RFLP analysis and amplicon sequencing. Traditional microbial methods were used parallelly for enumeration and cultivation, bacterial strains were identified by 16S rDNA sequencing.

Based on our results, fish arriving from the examined lake had a diverse microbial community. Cell counts dramatically decreased by the end of processing. During cold storage, vacuum package greatly affected the microbial community, while temperature influenced the cell count and shelf life of the

product. Amplicon sequencing revealed that the key species of the stored carp fillet are *Brochothrix thermospecta*, *Pseudomonas syringae* group, *Vagococcus fluvialis*, *Shewanella baltica* and *Acinetobacter celticus*. With additional cultivation, more than 200 bacterial strains were isolated and identified. By the time of writing, 11 species of 5 genera were identified as emerging opportunistic bacteria. The antibiotic resistance and virulence profiling of these strains are in process.

Our results clarified that several uncommon, presumably environmentally transmitted opportunistic bacteria are detectable during storage of common carp originating from the chosen fish farm. In the future, the characterization of these bacterial strains enables to find an effective solution for the prolonged storage and safety of freshwater fish.

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**P 023 Spatial and temporal variation of microbial communities, faecal indicators and physicochemical parameters: characterising water sources in a mixed-use agricultural catchment in Sydney, Australia**

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Recent studies have confirmed direct links between pathogenic microorganisms in agricultural water sources and disease outbreaks associated with fresh produce consumption. A notable example is the 2018 *Escherichia coli* O157:H7 romaine lettuce outbreak in Yuma, Arizona, which infected 210 people and resulted in five deaths. Further investigation into the microbial status of our water sources, and the preceding environmental conditions linked to the detection of pathogenic bacteria, will help us to assess their potential to cause similar outbreaks. This study profiles the microbial populations present in the water and sediment of a mixed-use, peri-urban agricultural catchment in Sydney, Australia. Samples were collected over multiple seasons and at various points upstream and downstream of agricultural activities. Total coliforms and generic *E. coli* were quantified using the IDEXX Colilert® method. Both common (e.g. pH, turbidity) and more specific (e.g. fDOM, chlorophyll *a*) physicochemical water quality parameters were recorded. DNA extracts from the environmental samples were amplified for the V3-V4 region of the 16S rRNA gene and used for bacterial community profiling. Statistical modelling was used to draw out spatial and temporal patterns in the abundance and biodiversity of the microbial populations and to determine significant relationships to water quality parameters. Preliminary results indicate variation in faecal contamination loading between sites and between seasons. Additionally, there are marked differences between microbial communities present in the water column and in the sediment. This study is unique because it involves the collection of a substantial amount of metadata that will help to explain the variation in microbial abundance and diversity and place the microbial profiles in context of the surrounding geography. Future work on this agricultural catchment includes utilising real-time PCR to identify classical and emerging human pathogens in the water sources that are of relevance to the fresh produce industry.

**P 024 Status of water sources, hygiene and sanitation and its impact on the health of households of Makwane Village, Limpopo Province, South Africa**

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The key to reducing or even eradicating the burden of waterborne diseases is through appropriate sanitation facilities and piped water systems. Installation of centralised system may take decades to be established, especially in impoverished rural communities of African countries. The current study aims to generate information on the health status of rural communities and provides critical information, which policy makers may consider in order to meet the basic human needs of this community. Results

of the survey revealed a complete absence of improved drinking water sources in the community (100%). High percentage of households not treating water (81%) prior to use, 41% lacking access to improved sanitation facilities and 65% do not allow children under 12 years old to use the toilets. 86% households practice open defecation as an alternative sanitation facility. Stream was the main water source for the community (31%). In terms of health outcomes, the most prevalent health problem was found to be diarrhoea (75%), which occurred mostly in children less than 5 years old and was found to persist up to 3 days (34%). The community generally visited the clinic (75%) in cases of health problems. This study highly recommends the implementation of point-of-use household drinking-water treatment in Makwane households for the production of safe drinking water. Furthermore, community-based hygiene education is recommended in terms of drinking water storage, cleaning of container-stored water and safe disposal of wastewater. Homes and the surrounding area must be adequately cleaned on a regular basis to eliminate flies. Open defecation in this community should be discouraged to mitigate bacterial contamination of water sources and transmission of diseases.

#### **P 025 Method development for enteric virus recovery from primary sludge**

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Enteric viruses are a leading cause of gastroenteritis, and environmental surveillance of enteric viruses in wastewater helps to estimate disease burden, identify 'silent' outbreaks, and plan vaccine interventions. However, some at-risk locations utilize pit latrines rather than water-based sanitation systems. The objective of this study was to develop a protocol for enteric virus concentration and recovery, for use with pit latrine waste. Primary sludge was used as a surrogate for latrine waste, and poliovirus type 1 (PV1) recovery was optimized. Samples were dosed with PV1 into 100 mL primary sludge. Samples were initially processed by acid adsorption and/or centrifugation. The solid and liquid fractions were then processed separately. The solid fraction was eluted three times, concentrated via skimmed-milk flocculation, and extracted twice with Vertrel™. The liquid fraction was concentrated by skimmed-milk flocculation, and extracted twice with Vertrel. In a portion of samples, the solid and liquid fractions were combined prior to skimmed-milk flocculation and extraction. PV1 recovery was quantified by plaque assay. Acidification increased PV1 recovery from the solid fraction (48.2 vs. 23.9%;  $p=0.034$ ) and decreased PV1 recovery from the liquid fraction (12.4 vs. 42.7%;  $p=0.0004$ ), yielding an average total recovery of 60.6% for acidified and 66.6% for non-acidified samples. For acidified samples, PV1 recovery from the solids using Elutions 1 and 2 (23.1% and 21.0%, respectively) was significantly greater than for Elution 3 (4.3%;  $p=0.015$ ,  $<0.001$ , respectively). Recovery from the first extraction (37.9%) was significantly greater than from the second extraction (10.3%;  $p=0.004$ ). When the processed solid and liquid fractions were re-combined prior to skimmed-milk flocculation, PV1 recovery was lower than if these sample portions were processed separately. This study demonstrated optimized PV1 recovery from primary sludge with separate processing of the solid and liquid fractions. Future work should include field-testing with latrine waste and surveillance of additional targets.

#### **P 026 Prevalence of opportunistic pathogens in school building premise plumbing systems during periods of low use and a transition to normal use**

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The spread of opportunistic pathogens via building water supply and premise plumbing systems is of public health concern. This study was conducted to better understand microbial water quality changes in a LEED-certified school building during low water use (Summer) and normal water use (Autumn). The copper plumbed building contains water saving devices, hot water recirculation system, and

receives chloraminated drinking water from a public water system. Six sampling events at 20 in-building locations were completed from June to October 2018: 3 before students returned to school, and 3 after. *Legionella* species (spp.), *Legionella pneumophila*, *Mycobacterium* species, *Mycobacterium avium*, *Acanthamoeba* species and *Naegleria fowleri* were enumerated by quantitative PCR. Cell culture was also used to detect *Legionella* spp. in water and biofilm samples. Water collected after the water meter had different pH, disinfectant residual, Heterotrophic Plate Counts (HPC), and metals characteristics than water deeper in the building. A high occurrence of *Legionella* spp. (100%, concentrations ranging from 54 to 1.7E+05 gene copies/100mL) and *Mycobacterium* spp. (100%, concentrations ranging from 430 to 2.2E+07 gene copies/100 mL), lower occurrence of *Mycobacterium avium* (68%, concentrations ranging from 1.1E+03 to 2.1E+06 gene copies/100mL) were observed throughout the water systems according to quantitative PCR. Two species of amoeba were also detected. *Legionella pneumophila* was not detected in any of the samples. Culturable *Legionella* were detected in water samples collected during periods of low water use. DNA sequencing showed that the isolates are closely related to *Legionella donaldsonii* and *Legionella tunisiensis*. There was a statistically significantly difference in concentrations of both *Mycobacterium* and *Legionella* between school breaks and when school was in session, but there was no significantly difference in concentrations between different sampling locations of the school for either *Mycobacterium* or *Legionella*. Recommendations for school water sampling plan design, water quality investigations, and remediation will be described.

**P 027 Development the simple, specific and sensitive method to detect *Biomphalaria glabrata*, the intermediate host snail of *Schistosoma mansoni*, by applying environmental DNA approach**

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Schistosomiasis, one of the most important neglected tropical diseases that more than 200 million people were estimated in need of treatment in 2016, is caused by parasitic trematode (schistosomes) of the genus *Schistosoma*. In their life cycle, schistosomes need development phase in intermediate host snails before acquiring the infectious ability to human. To prevent schistosome infection, WHO stated that improving access to clean water and reducing the number of snails are the comprehensive countermeasures. For snail control, it is essential to clarify their spatio-distribution. To this aid, ecological surveys by visual searching intermediate host snails has been performed although time and labor consuming. This research aims to establish the simple, specific and sensitive method to detect schistosomes and their intermediate host snails by applying environmental DNA (eDNA). The eDNA is a molecular imprint of inhabiting species in the natural environment. It includes microorganisms themselves and extracorporeal DNA derived from macroorganisms. Once collecting several litters of water sample, we can predict the presence/absence of the target organisms by such as target-specific PCR and metagenomics approaches. In this study, we determined the optimal condition to concentrate eDNA by filtering method changing water volume, filter material, filtration pressure. The specific primer pairs for *Biomphalaria glabrata*, intermediate host snail of *Schistosoma mansoni*, were also determined. The aquarium water, in which breeding *B. glabrata*, was used for investigation. The filter material did not affect the concentration performance of eDNA. Low filtering pressure (20 kPa) gave the high recovery of eDNA in comparison to high pressure (90 kPa). Among the published primer pair, GITS2F and GITS2R showed specific detection of *B. glabrata* by PCR, and using this, *B. glabrata* eDNA could be detected from 100 mL of aquarium water. Thus we could establish the prototype of *B. glabrata* monitoring method by eDNA approach.

**P 028      Detection and characterization of *Staphylococcus* from drinking water fountains and mist makers in public parks in the São Paulo city, Brazil**

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Public parks are composed by green areas presenting recreational role, enhancing environmental quality in the cities. Park visitors use these spaces to practice physical activities, social quality time among other activities, being a democratic space. The social role of these spaces in urban context is to provide improved conditions for their visitors including drinking water quality. Usually, drinking water from fountains and mist makers in municipal parks are supplied by the public distribution system. Worldwide, drinking water microbiological parameters do not cover opportunistic pathogens, such as *Staphylococcus* genus, which is responsible for the second largest number of infections in humans. Considering the importance of parks in urban space, the aim of this study was to detect the presence of *Staphylococcus* species in water samples from drinking water fountains ( $n = 468$ ) and biofilms from mist makers ( $n = 84$ ) at four municipal parks of São Paulo city spanning thirteen months. The detection of *Staphylococcus sp.* was performed according to the *Standard Methods for the Examination of Water and Wastewater* (23nd, 2017). The characterization of the isolates was carried out by the detection of species-specific genes for *S. aureus* (genes *nuc* and *coa*) and for *S. epidermidis* (gene *RecN*). The presence of virulence factors was evaluated by detecting the genes *sea*, *seg*, *luk-PVL* and for the biofilm formation by detecting *icaAB* gene. Out of 552 samples, 22% were contaminated with *Staphylococcus*. *S. epidermidis* was present in 31%, followed by 19% of *S. aureus*. The results reveal the importance of surveillance of drinking water fountains and mist makers since there is potential risk to human health.

**P 029      Genome amplification for monitoring the presence of human pathogens in water treatment processes, a longitudinal study**

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While the levels of hygienic indicator bacteria in water treatment processes are determined regularly, the burden of human enteric pathogens usually remains unknown. Genome amplification could offer a technique for monitoring the presence of infectious microbes and for assuring drinking water hygiene. The main objective of this study was to determine the presence of human pathogens in processes of water treatment plants and wastewater treatment plants in Finland during a one-year survey in 2017/2018. There are in total 230,000 residents in the water supply networks studied. The water treatment plants use both surface water ( $N=4$  lake) and ground water ( $N=4$ ) as a source for drinking water. Surface water and groundwater treatment processes varied and include, for example, coagulation and flocculation, clarification, sand and activated carbon filtration, aeration and chemical oxidation. The wastewater treatment plants ( $N=4$ ) use conventional activated sludge process for removal of suspended solids, organic matter, phosphorus and nitrogen. Treated wastewater is then discharged to natural water sources.

The sampling frequency was on the average once per month from September 2017 to September 2018 (in total 349 samples). Several concentration methods were applied and evaluated for the detection of pathogens in water treatment processes, including dead-end ultrafiltration, monolithic affinity filtration and Centricon Plus filters. Norovirus, rotavirus, enterovirus, parechovirus and rhinovirus were detected with a real-time reverse transcription-PCR assay. A next generation sequencing (NGS) method was applied to sequence viruses (virome), bacteria (16S gene region) and parasites and fungi (18S gene region). Following further validation, these techniques have the substantial potential to extend and speed up the monitoring of water microbial quality in the future.

Key words: Next generation sequencing (NGS), pathogen, real-time PCR (RT-PCR), wastewater, water.

#### P 030 Multilocus genotyping of *Giardia Duodenalis* in Clinical and Environmental Samples

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**Background:** The transmission of *Giardia* occurs through the fecal-oral route by exposure to contaminated water and food. Few studies have addresses the assemblages of *Giardia* circulating in the population and the environment in Israel.

**Aims:** This study was performed to determine the assemblages and sub- assemblages of *Giardia* present in stool samples compared with sub-assemblages in wastewater.

**Materials & Methods:** DNA was extracted from 46 *Giardia* positive stool samples and 35 raw wastewater samples after Immuno-magnetic separation (IMS) and microscopic enumeration. *Giardia* was detected by PCR and nested-PCR using  $\beta$ -giardin and glutamate dehydrogenase genes. While the PCR-RFLP was applied to determine the assemblage present in the samples. Sequence analysis of  $\beta$ -giardin was performed to determine the sub-assemblages. *Giardia* cysts concentration in stool samples ranged between  $1 \times 10^2$  to  $1.4 \times 10^7$  cysts/gr, whereas the concentration of *Giardia* in wastewater ranged from  $1.1 \times 10^2$  to  $9.1 \times 10^3$  cysts/liter.

**Results & Conclusions:** Assemblage B was identified in 36 out of 46 (78.2%) stool samples, sub-assemblages B, BI, BIII and BIV were identified by sequencing. Only 10 out of 46 (21.7%) stool samples were identified as assemblage A (sub-assemblages A, AII and AIII). It is interesting to note that, all of the tested raw wastewater samples were positive for *Giardia* cysts assemblage A. The Nested-PCR products of  $\beta$ -giardin gene were sequenced and the sequences were subjected to phylogenetic analysis. Good agreement was observed between RFLP using the  $\beta$ -giardin gene and the sequence analysis. The results of this study indicate the usefulness of the methods for the classification of *Giardia* isolates in stool and wastewater samples and these methods will be useful to determine the source of *Giardia* in another environmental samples. To our knowledge this is the first attempt to identify *Giardia* assemblages in clinical and environmental samples in Israel.

## **P 031 Fungal and mycotoxin prevalence in treated drinking water distribution system**

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Occurrence of fungi in treated drinking water has serious implications to human health, and is of paramount importance to urban water distribution systems. Fungi have been least considered and discussed in the monitoring of waterborne pathogens in treated drinking water supply systems. Fungi and their metabolites are however, now regarded as an emerging cause of public health and water quality concerns due to impacts such as mycotoxicosis, allergies, meningitis and dermatitis. This study was carried out to investigate the prevalence of fungi in treated drinking water in selected areas of Johannesburg West, South Africa. Water samples were collected over period of one year from 30 identified sites. Culturing for fungi was done by plating aliquots of filtered water samples on potato dextrose agar and incubating at 28°C for 4-7 days. Fungal isolates obtained were characterized by DNA sequencing of the internal transcribed spacer (ITS1 and ITS4). The partial ITS sequences of isolates obtained were subjected to hierarchical clustering to obtain the operational taxonomic units, based on a taxa cut-off set at 97% similarity. Fungi were positively detected at most of the sampling sites for the duration of the sampling period. All isolates characterized belonged to the phylum Ascomycota. The most common genera were *Aspergillus* (20% of isolates), *Penicillium* (16%), *Trichoderma* (9%), and *Purpureocillium* (10%). Other genera isolated included *Fusarium*, *Alternaria*, *Coniothyrium*, *Cladosporium*, *Sarocladium*, *Exophiala*, *Auerobasidium*, *Arthrinium*, *Meyerozyma*, *Phoma*, *Talaromyces*, and *unclassified Nectriaceae and Dothidiomycetes*. Most of the identified fungal species are also known to produce mycotoxins that are related to public health complications, with *Penicillium* species having the most identified mycotoxins followed by *Aspergillus* species. These findings prove the need to include fungi in the assessments of microbiological contaminants of public health concern in public water supplies.

**Keywords:** Fungal prevalence, mycotoxins, treated drinking water, water contaminants, public health impacts

## **P 032 Detection of Shiga Toxin-producing *Escherichia coli* (STEC) and other pathogenic *E. coli* in small community water supplies in Puerto Rico**

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Shiga toxin is one of the most potent bacterial toxins known, and is produced by some serotypes of *E. coli*, specifically those belonging to the enterohemorrhagic *E. coli* (EHEC) pathotype. The toxin can cause bloody diarrhea, hemolytic uremic syndrome (HUS), kidney failure and death. In the United States it is estimated that Shiga toxin-producing *E. coli* (STEC) causes more than 265,000 infections annually, as a result of ingestion of contaminated food or water. The aim of this study was to investigate the occurrence of Shiga toxin encoding genes, *stx* (*stx1* and *stx2*) in source and distributed water samples collected from small potable water systems in Puerto Rico. Seven systems were visited during two sampling campaigns in 2015 and 2016. Fifty-two percent (52%) of the samples tested by multiplex PCR (mPCR) were positive for the presence of the *stx* genes, this percentage was higher within the raw water samples. The *stx2* gene was more abundant than *stx1*.

Portions of the samples were enriched with total coliform PA medium to test possible changes to STEC procedures. Six enriched samples that were negative to TC, *E. coli* or TC and *E. coli* were *stx* positive by mPCR. These results might indicate either the presence of the gene in the sample matrix

(cell-free DNA), the presence of other bacteria carrying a similar gene, or that the counts were below the limit of detection of our culture-based assay. Enrichment provides an alternative protocol for detection of STEC that would likely be missed by the standard total coliform assay and might be tested as an alternative to culture techniques. The presence of *stx* encoding genes in surface water and distributed water from all 7 sampled systems and suggests STEC as a potential health risk in the over 148,000 systems in the USA and its territories.

**P 033 Microorganisms from surface waters contribute to the inactivation of human echovirus 11: toward biocontrol of viral pathogens?**

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Human viruses are widespread as contaminants in surface waters and represent a significant health risk. Viral pathogens can persist in aquatic ecosystems and cause infections via contaminated water or food. Among the different factors governing the environmental persistence of enteric viruses, removal by indigenous microorganisms, in particular predation by protists, has received little attention to date. This study aims to determine the contribution of indigenous protists to the inactivation of human viruses in different surface waters. Incubation of human echovirus 11 (E11) in water from Lake Geneva and seawater led to an inactivation of 2.5-log within 48 hours at 22°C, whereas inactivation in sterile controls was minor (0.8-log reduction). This inactivation was mainly attributed to the action of protists in the eukaryotic fraction of the samples. The inactivation of viruses was shown to be temperature-dependent, with a complete inhibition of biological inactivation at 8°C. In addition, inactivation depended on both the species of virus and protist species. Among three protist isolates tested (*Paraphysomonas* sp., *Uronema marinum* and *Caecitellus paraparvulus*), *Caecitellus paraparvulus* was particularly efficient at removing E11 (2.1-log reduction over four days with an initial protists concentration of 1000 cells / ml). In conclusion, this study suggests that indigenous protists present in lakes and oceans are important biological contributors to the inactivation of E11. These results pave the way for further research to better understand how protists control human viral pathogens in aquatic ecosystems and how microbial inactivation could be exploited as a water treatment solution to enhance microbial safety.

**P 034 Determining Norovirus Infectivity Based on Specific Detection of Negative Strand Viral RNA**

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**Introduction**

*In vitro* cultivation of human norovirus (HuNoV) using stem-cell derived human intestinal enteroids (HIEs) has been reported recently, which potentially allows determination of HuNoV infectivity in water samples. The genome of norovirus is a positive-sense single-stranded RNA, which acts as a template for genome replication via the synthesis of a negative strand RNA intermediate. The synthesis of negative strand RNA serves as a marker for the active replication of norovirus. In this study, we aimed to develop a methodology to determine norovirus infectivity based on specific detection of negative strand RNA replication intermediate.

**Materials and Methods**

HuNoV GII.3 and murine norovirus (MNV) were inoculated to HIEs and RAW 264.7 cells, respectively. Viral RNA was extracted from the infected cells and the number of negative strand RNA was quantified using strand-specific RT-qPCR. Synthetic positive- and negative strand RNA molecules were used to validate strand specificity of the RT-qPCR.

## **Results**

Strand-specific quantification was achieved using specific RT primers tagged with a non-viral sequence at the 5' end, and no non-specific reaction with uninfected cells was observed. For both HuNoV and MNV, the numbers of negative strand RNA in infected cells substantially increased as a result of viral replication in host cells. Detectable increase in copy numbers of negative strand RNA was observed before the development of cytopathic effect, suggesting the possibility of fast and sensitive viral infectivity determination using the proposed strand-specific RT-qPCR method combined with *in vitro* cultivation.

## **Conclusions**

We developed a negative strand-specific RT-qPCR procedure for determination of norovirus infectivity. This method is highly sensitive, likely because a number of negative strand RNA can be produced from one infectious virion during *in vitro* replication. This approach potentially contributes to elucidating the behavior of infectious HuNoV particles, including occurrence in environmental samples, environmental persistence, and susceptibility to disinfectants.

### **P 036      Detection of noroviruses in environmental samples using digital reverse transcription (RT)-dPCR – a comparison with real-time RT-qPCR**

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**Background:** For detection of human noroviruses and other enteric viruses, real-time reverse transcription (RT)-PCR is a widely used method due to its sensitivity, practicality, reproducibility and rapidity. In recent years, digital approaches of PCR have been introduced. Their advantage is that quantification is not based on standard curves but that absolute values are directly given for each sample.

**Aim:** Our aim was to test nucleic acid identification of norovirus using digital (dPCR) and real-time quantitative (qPCR) amplification methods. In this pilot study, we tested artificially and naturally contaminated samples.

**Methods:** We analysed frozen viral RNA control samples as well as 34 nucleic acids that were extracted with NucliSENS® miniMag® system from water, sewage, surface swabs or food. For RT-qPCR, primers and a fluorescence-labeled probe specific for norovirus GI or GII according to ISO 15216 and QuantiTect probe RT-PCR kit (Qiagen) were used. For RT-dPCR, the same primers and probes as in the qPCR were applied together with One-Step RT-ddPCR with Advanced Kit for Probes (Bio-Rad) in droplet digital PCR Bio-Rad system.

**Results:** Our experience was that dPCR was comparable to qPCR when GII norovirus was tested. A relative sensitivity of 93 % for GII norovirus was observed for RT-dPCR; for GI norovirus it was lower. When naturally contaminated sewage samples were tested, 6.9 x fold differences (1.1 - 18.6 x fold) were observed in norovirus GII genome copy quantities, RT-dPCR giving a higher value than RT-qPCR in each case.

**Conclusions:** Digital PCR is a promising technique providing an accurate quantification and it seems that an existing qPCR assay can often be easily modified to a dPCR assay. Still, more user-friendly applications and cheaper devices would be welcome.

**P 037      Emerging opportunistic pathogens in artificial water bodies**

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Aquaculture is a dynamically growing agri-sector with priorities defined by the National Aquaculture Strategy. The primary goals determined by this strategy can be reached when the productivity and safety of fish farming is ensured. The aim of this project is to determine the critical biological factors in fish product line 'from fish ponds to the table'. Classical microbiological methods were used to determine the microbial population of fish ponds. Water and sediment samples of fish ponds were collected in 2017 and 2018, during spring and autumn periods from influent, effluent water and the water body of 6 fish ponds in Hungary. The surface of a group of farmed fish (*Cyprinus carpio*) were also sampled for bacterial cultivation. Nutrient and selective/differential media were used for cultivation, pure cultures of discrete colonies were identified based on 16S rDNA. From the established strain collection cultures of emerging opportunistic pathogens, such as members of genera *Acinetobacter*, *Aeromonas* and *Pseudomonas*, were used for further examinations. Antibiotic resistance was determined with Etest strips based on the determination of minimal inhibitory concentrations (MICs) according to the recommendation lists of CLSI and ECDC. Virulence factors, such as haemolytic activity on Columbia agar plates, were also determined.

Based on the results of our examinations, the widely known nosocomial pathogen *Pseudomonas aeruginosa* have a low abundance in surface water environments (5.4%), with a sensitive antibiotic resistance phenotype. However, the phenotypic manifestation of virulence and the antibiotic resistance against several agents may be expected among other emerging pathogens originating from artificial water bodies. The dominance and the role of *Aeromonas* spp. with some verified virulence traits is in a great interest in the future.

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**P 038      Occurrence and identification of *Cryptosporidium* and *Giardia* from surface water catchment in São Paulo, Brazil**

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Review studies on waterborne outbreaks have been showing that *Cryptosporidium parvum* and *Giardia duodenalis* are the primarily responsible for the highest number of the cases recorded worldwide. Contamination of surface waters catchments by these protozoa is a risk factor to human health because both parasites are resistant to chlorination, which is a conventional process used for disinfection in drinking Water Treatment Plants (WTP). The present study aimed to identify species of *Cryptosporidium* and *Giardia* recovered from surface water catchment samples from 11 municipalities from the State of São Paulo, totaling 128 samples. Quantification of both parasites was carried out according to method 1623.1 (USEPA, 2012). In order to identify parasites, the recovering of (oo)cysts from slides followed USEPA's protocol by scraping slides, then Real Time PCR using the 18S rRNA genes for *Cryptosporidium* and SSU for *Giardia* were carried out. Results showed that the frequency of occurrence of these protozoa at the catchment points was 29,7% for *Giardia* and 30,4% for *Cryptosporidium*. Cysts were present in 10 of 11 catchments points with frequencies varying from 17 to 100% with concentrations ranging from <0.1 (Limit of Detection=0.1) to 17.7 cysts/L, which put in evidence the low quality of water. Oocysts occurred in a frequency from 17 to 50% in positive samples with concentrations ranging from <0.1 (LD) to 11.5 oocysts/L.

The real-time PCR assay detected a total of 81.4% (57/70) of positive samples for *Cryptosporidium hominis-parvum-meleagridis* and 21.4% (15/70) samples positive for *Giardia duodenalis*. The frequency of occurrence of these pathogens and the high concentrations of anthropic species found is a concerning scenario for human health, considering samples have been collected from catchment sites for public water supply.

**P 039      Presence of *Legionella pneumophila* in household drinking water reservoirs of two cities of northeast of Argentina**

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**Introduction.** *Legionella pneumophila* is considered an emerging pathogen that could be found in a wide diversity of aquatic environments. It implies a public health risk when it colonizes man made water systems and grow to high concentrations. Resistencia and Corrientes are two cities localized in the northeast of Argentina and the Paraná River is the main water supply for both of them.

**Objetive.** The aim of this study was to detect and compare the prevalence of *Legionella pneumophila* in household drinking water tanks of Resistencia and Corrientes, two cities that share the same water source.

**Methods.** The sampling was non-probabilistic and the samples taken were a total of 50, 30 from Resistencia and 20 from Corrientes. The protocol utilized for *Legionella* isolation was culture, following the recommendations of the ISO standard 11731:2017. A qPCR assay was performed to all positive cultured samples identified as *Legionella* spp. The genomic targets used were the 23S rRNA gene and the *mip* gene to corroborate the genus and to identify *L. pneumophila*, respectively.

**Results:** Out of the 50 water samples analysed, 14 were positive for *L. pneumophila* (14/50; 28%) for both cities. Among positives, 11 were from Resistencia (11/30; 36.7%) and 3 were from Corrientes (3/20; 15%). In addition, 3 samples of Resistencia (3/50; 6%) were also positive for another species of the genus *Legionella*.

**Conclusions:** Our exploratory study showed the presence of *L. pneumophila* and other *Legionella* species in residential drinking water reservoirs of the two cities. Although, Resistencia and Corrientes cities share the same source of water supply, they seem not to share the same frequency of *Legionella* species. This result could be related to the presence of residual free chlorine in water tanks. A greater number of samples should be analysed, including the water source, in order to obtain accurate conclusions.

**P 040      Combining flow cytometric and taxonomic analysis of bacterial community dynamics to study the biostability of drinking water resources – Part 1: Flow cytometric determination of microbial cell numbers**

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Over the past years, the issue of biostability of water has gained increasing attention. Re-growth of unwanted bacteria may deteriorate taste, odour and appearance of drinking water; growth of facultative pathogens such as *Pseudomonas aeruginosa* is of particular concern for public health. The aim of this project was to develop a standardized protocol for the determination and interpretation of

bacterial re-growth dynamics in ground- and drinking water based on flow cytometry and taxonomic community analysis by high-throughput 16S rRNA gene amplicon sequencing (see also joint contribution by Savio et al.).

Groundwater and drinking water samples from a riverbank filtration system were collected from sites in different distance to the river. Biostability was determined by incubating the samples in batch at 10°C for three weeks, simulating water stagnation during storage and distribution in the supply network. At daily to weekly intervals, the increase in microbial cell numbers was determined by flow cytometry and compared to the changes in heterotrophic plate counts obtained by the traditional cultivation method. Additionally, the influence of different incubation temperatures on bacterial growth rate and yield was investigated and the reproducibility of the method assessed by a series of parallel incubations.

An innovative protocol for the determination and interpretation of biostability was established. Samples from sites in close vicinity to the river showed much higher dynamics and inter-sample variability than water from remote sites. Surprisingly, bacterial populations incubated at higher temperatures did not demonstrate a higher growth compared to standard temperature incubations at 10°C.

Future work will focus on the influence of the biogeochemistry on the bacterial re-growth potential using the established protocols. Moreover, automated flow cytometry tools enabling a higher time resolution of bacterial growth shall be applied, supporting a reproducible and straightforward determination of biostability and its prediction in ground- and drinking water.

**P 041 Combining flow cytometric and taxonomic analysis of bacterial community dynamics to study the biostability of drinking water resources – Part 2: High-throughput 16S rRNA gene amplicon sequencing**

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Over the past years, the issue of biostability of water has gained increasing attention. Re-growth of unwanted bacteria may deteriorate taste, odour and appearance of drinking water; growth of facultative pathogens such as *Pseudomonas aeruginosa* is of particular concern for public health. The aim of this project was to develop a standardized protocol for the determination and interpretation of bacterial re-growth dynamics in ground- and drinking water based on flow cytometry and taxonomic community analysis by high-throughput 16S rRNA gene amplicon sequencing. To investigate the composition and dynamics of bacterial communities in ground and drinking water, two different library preparation methods for high-throughput sequencing of 16S rRNA gene amplicons have been evaluated for representative water samples from a riverbank filtration system. To study the bulk bacterial communities, a widely used protocol targeting the V3-V4 variable region of the bacterial 16S rRNA gene (primer pair Bakt\_341F & Bakt\_805RN) has been evaluated and optimized. To evaluate the methods' specificity in regard to the largely unknown groundwater microbiome, selected samples were additionally processed using an alternative library preparation protocol (V1-V2 region of the 16S rRNA gene; primers 8F & 338R).

For the specific detection of potential facultative pathogenic populations within the genus *Pseudomonas*, a recently published protocol for the high-resolution study of this taxonomic group has been evaluated (primers Pse434F & Pse665R). DNA libraries were sequenced on an Illumina MiSeq Sequencer applying both v2 (2 x 250 bp) and v3 (2 x 300 bp) chemistry. Following the final evaluation of the library preparation protocols, bacterial community dynamics will be analysed for a comprehensive batch-culture experiment (*cf.* contribution by Vierheilig et al.). While the genus *Pseudomonas* has been selected as model target group to generally evaluate the detectability of opportunistic pathogens in alluvial groundwater resources, future work will focus on other pathogenic bacteria of public health concern.

## **P 042 Proliferation potential – Drinking water: Standardization and quality assurance**

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The biostability of drinking water is an essential quality criterion for storage and distribution. The supply of nutrients in drinking water, treatment processes (in particular oxidative disinfectants), temperature and residence time in the distribution system are important factors. Methods for the determination of biostability are e.g. the assimilable organic carbon (AOC), the biodegradable fraction of dissolved organic carbon (BDOC) and the determination of the proliferation of water bacteria. There are large differences between these methods, which lead to incomparable results and assessment. The proliferation potential of drinking water serves as a valuable basis for decision-making and problem solution in water treatment and distribution.

A standardized method to determine the proliferation potential was developed as follows: All glass bottles were muffled at 550°C to remove the carbon. The water samples were taken in 2-liter-glass bottles and - after arriving in the laboratory - immediately filled into six storage vessels (250 ml glass bottles) pre-rinsed with sample. The storage took place at a temperature of 10±2°C, which roughly reflects the temperature in Austrian water distribution systems. Colony counts were determined on the day of sampling, after 7 days and after 14 days of storage by two methods: ISO 6222 (22±2°C / 3 days) and R2A medium (27±2°C / 7 days). Pooled samples of three storage vessels were analysed in triplicate.

It was demonstrated that single analysis of single vessels resulted in highly variable colony counts; triplicate analysis from pooled samples are therefore necessary. The storage time of 14 days led to more meaningful results and higher precision (coefficients of variation: 7 days 34%; 14 days 14%). This standardized procedure is also suitable for further flow cytometric analyses and molecular methods.

This is a joint investigation of the Interuniversity Cooperation Centre for Water & Health ([www.waterandhealth.at](http://www.waterandhealth.at)).

**P 043 Evaluation of a Methodology for Virus Recovery from Solid Waste Landfill Leachate**

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The leachate is a liquid formed from solid waste landfill and a serious public health problem with high potential of contamination of groundwater and surface waters. The aim of this study was to establish the best methodology for PP7 bacteriophage efficiency recovery from solid waste landfill leachate and possible application in environmental monitoring of viruses in leachate. Three samples of leachate were collected from October 2018 to February 2019 in one landfill located in the state of Rio de Janeiro, Brazil, and spiked with  $1,22 \times 10^6$  genomic copies of PP7. The samples were tested with two different protocols for virus concentration: (1) flocculation method using skimmed milk and (2) ultracentrifugation method. After concentration, nucleic acid was extracted using *QIAamp Fast DNA Stool mini kit®* and submitted to reverse transcriptase reaction. Samples were quantified by qPCR and PP7 recovery efficiency mean was 0.010% using the flocculation method and 9.13% by ultracentrifugation. Once we found an efficient recovery method, ultracentrifugation, we started an environmental monitoring of human adenovirus (HAdV) in leachate collected in one landfill in Rio de Janeiro. HAdV was detected by qPCR in all analyzed leachates, with a mean of  $3.44 \times 10^6$  genomic copies/mL. Positive samples by qPCR were amplified by conventional PCR and visualized on agarose gel electrophoresis. Samples were purified and sequenced by Sanger' method for molecular characterization. Two samples showed 96.34% nucleotide similarity to human B adenovirus (GenBank: KF268124). The other sample showed 96.52% similarity with human adenovirus 41 (GenBank: KY498757). As shown by our study the establishment of methodologies for detecting viruses in leachate could represents an innovative approach for virus detection, and HAdV monitoring could in the future determine health risk of workers who carry out activities managing solid waste and other populations exposed to different environmental matrices contaminated by this effluent.

**P 044 Zero, zilch, nada: Unadulterated microbial non-detects prevent bias**

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When microorganisms such as waterborne pathogens are not detected in a sample, the concentration has conventionally been reported as a value below a detection threshold such as a method detection limit. These values are mathematically interpreted as "censored" measurements of microbial concentrations. This has led to extensive debate in quantitative microbial risk assessment (QMRA) concerning how to handle these data when investigating risks posed by pathogens in drinking water and food. Drawing upon approaches used in the analysis of chemical non-detect data, there has been increased attention in QMRA to statistical methods tailored for censored non-detects. But are these data truly censored?

This work asserts that microbial non-detects in both enumeration- and detection-based methods are not correctly interpreted as censored data; rather, they are counts and corresponding unbiased concentration estimates of zero. These results are uncertain due to known random measurement errors in the same way as non-zero counts or most probable number assays. It is bizarrely inconsistent and inherently biased to view a non-detect result for discrete microorganisms with great skepticism while

simultaneously regarding a concentration estimate arising from a count of one as an exact measurement of concentration.

A set of *Giardia* count data from a raw drinking water source is analyzed using a suite of data analysis approaches. Commonly used substitution approaches can lead to inflated estimates of mean concentrations and risks, but so can approaches appropriate for censored data when the data are misrepresented as censored. This bias can lead to misinformed infrastructure and operational decisions. We propose that raw microbial counts or presence/absence patterns be reported with the corresponding sample volumes to avoid misinterpretation of these data as censored microbial concentrations and to facilitate appropriate data analysis using approaches that incorporate available knowledge about random errors associated with these detection- and enumeration-based microbial methods.

#### **P 045      Continuous surveillance of microbial water quality by automated and online flow cytometry**

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bNovate Technologies

One of the major challenges in ensuring safe drinking water today is the difference between the time it takes to produce, distribute and consume the water, and the time it takes to analyze it, which can differ by several days. In the drinking water industry, real-time monitoring of the water quality mainly relies on the measurement of physicochemical parameters such as turbidity, pH, conductivity or total organic carbon. The microbiological testing generally used for surveillance are of limited use and does not allow any operational adjustments prior to public water supply. Indeed, the traditional plate counting, routinely used for enumerating the total heterotrophic bacteria and fecal bacteria, suffers from very low throughput and lengthy analysis time (3 to 5 days). By the time routine microbial analysis reveals a possible bacterial contamination, the investigated water will often have already been distributed and consumed. Therefore, faster and automated microbiological monitoring tools are becoming essential. The development of flow cytometry methods for the rapid detection and characterization of microbial cells in drinking water has opened up some completely novel possibilities. In that context, we have developed the BactoSense, an online and automatic flow cytometer able to continuously monitor microbiological parameters including the concentration, size and viability of bacteria in only twenty minutes. The use of the BactoSense has been proven to allow fast and realistic assessment of water treatment processes and rapid quantification of microbial cells in drinking water distribution network, dramatically reducing the current measurement time from several days to just minutes.

#### **P 046      Acclimatize: A Resilient Model for Bathing Water Quality**

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Acclimatize is an interdisciplinary project carried out by researchers in University College Dublin and Aberystwyth University, funded by the EU Interreg Va Ireland-Wales programme. Acclimatize aims to identify and quantify the biological and geographical sources of faecal contamination of bathing waters in a large scale urban area and to assess the impact of climate change on water quality. This operation works in partnership with local authorities and national agencies to design and deliver new practical management and monitoring protocols. The immediate results will be used to develop real-time predictive models that will predict the effects of climate change on Irish coastal waters.

Over a two-year period, bi-weekly samples were taken from eight river systems and three designated bathing zones in Dublin Bay. The levels of faecal indicator organisms, *E. coli* and intestinal

enterococci, were enumerated and the source of faecal pollution was determined using microbial source tracking (MST). Multiple linear regression models were used to assess the impact of these river systems on these bathing water zones.

The results showed a significant correlation between faecal indicator levels in urban streams and two of the at-risk bathing zones. In addition, there was a significant correlation between the faecal indicator levels in these streams and the human MST marker. Therefore, small polluted streams, severely impacted by human faecal pollution, had a significant impact on bathing water non-compliance. These extensive water quality surveillance results will be used to develop an overall drainage model of the Dublin catchment area.

#### P 047 Molecular methods for pathogen detection in drinking water treatment

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Within the EU-project Aquavalens\* new molecular methods for a fast detection of microbiological risks have been tested. These methods for pathogen and indicator detection were tested in large drinking water systems under the conditions of routine laboratories. A monitoring program of 13 months in 4 large water systems in Great Britain, Germany, Denmark and Spain was performed, including the pathogenic microorganisms Norovirus, *Campylobacter*, *Giardia* and *Cryptosporidium* and the indicators *E. coli* and coliform bacteria.

For the detection of the parameters the newly developed molecular methods (qPCR, FISH) were compared to the classical cultural methods. The monitoring included monthly sampling and analyses of different water types in different treatment steps (raw water, process water, treated water).

For the detection, large volumes of 100 l to 1000 l of the different water types were concentrated. As primary concentration step, a dead-end-ultrafiltration-system (Rexeed® filters) was used, followed by a secondary concentration by centrifugation and nucleic acid extraction.

The concentration steps showed to be very effective in decreasing the detection limits. For qPCR-methods such a concentration step is necessary for clean waters (treated water) in order to decrease the detection limit.

The qPCR-methods were sensitive to inhibitory effects, especially in the raw waters, the application of quantitative FISH-methods was also problematic in these waters, as the enriched particles disturbed the microscopic evaluation. In contrast, the use of the new methods was not disturbed by these effects in treated water samples.

The use of these new methods combined with concentration steps showed to be effective in special cases for trouble shooting. So far, they are not in a state to replace classical methods for routine monitoring in drinking water, as they are not robust enough and not standardized yet.

\* „Protecting the health of Europeans by improving methods for the detection of pathogens in drinking water and water used in food preparation”, grant agreement no. 311846.

**P 048      Rapid enzymatic activity measurement as an indicator of microbiological contamination – Results after 6 years of validations and experiments in different applications**

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Water scarcity, Environmental protection, sustainable use of resources are the key issues of today. To tackle this problem, the world must move in the direction of a circular economy and reuse resources like water. This requires efficient processes and therefore effective process control and monitoring. With regard to the water cycle, one of the most important quality parameters, microbiological contamination, is not yet available as a process parameter. The contamination cannot be assessed at (near) real-time for efficient decision making. For safety reasons, therefore, the highest possible degree of contamination must always be assumed. As a consequence, water treatment processes, have to be oversized, disinfectant has to be overdosed, in order to meet the quality targets. As this is neither efficient nor sustainable, various approaches for rapid microbiological measurements are being developed to fill this gap.

The ColiMinder is a fully automated enzymatic activity analyzer providing results 15 minutes after sampling. The measurement technology and first laboratory measurements have been presented at HRWM 2013 in Florianopolis, Brazil. Since that time, about 300,000 measurements have been carried out in various applications, from drinking water to wastewater. The presentation gives an overview of the obtained measurement results, comparisons with other methods, possibilities and limitations of the technology and the measurement approach. The measured enzymatic activities are beta-D-glucuronidase (GUS) as a fecal indicator and alkaline phosphatase (ALP) as an enzyme, indicating the presence of living microorganisms in general. The results presented include bacterial growth and disinfection experiments, detection limits, measurement ranges and reproducibility. The calibration procedure is presented as well as the potentials of standardizing the enzymatic measurement approach.

**P 049      Taking action for a future-proof drinking-water supply in Bavaria, Germany**

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The Bavarian public drinking-water supply is relatively complex comprising a high number of public water supply plants compared to neighboring regions. Both the public and the political community desire to maintain this structure with more than 2 000 public waterworks of various sizes and, in some places, complex structures. According to national law, the public health offices are responsible for the surveillance of water supply plants. With the increase of national and European standards, the responsible authorities are continuously facing challenges to supply drinking water that complies with these standards.

In order to maintain a safe water supply our project aims to support public health offices in their surveillance. Additionally, we intend to gain more information on the existing deficiencies to set priorities for public health policy. Both steps are leading to a future-proof water supply.

A joint working group consisting of members of both health and water management administration, developed detailed inspection checklists for every part of the water supply system – from catchment to costumer. The checklists are designed in a modular fashion to enable the surveillance authorities to put together individual test documents according to the structure of the relevant water supply plant or the suspected problem. To ensure the manageability during local inspections, the most important technical information and legal rules have been included in an explanatory part of the checklists.

In a pilot study, ten public health offices tested the checklists for practicability in various water supply plants. Currently all 76 public health offices are asked to supplement their annual monitoring of all water supply plants distributing > 1 000 m<sup>3</sup>/day or supplying > 5 000 people using the inspection checklist focusing on the operation and organisation of water supply plants.

## P 050 Fungal contamination of drinking water supplies

Memory Tekere

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Fungi in drinking water are considered an emerging problem and have no regulation standards set for their occurrence in many countries. Health concerns that arise from the contamination of drinking water by fungi are that some fungi are pathogenic, mycotoxicogenic and allergens, and also result in odors, off tastes and turbidity in water. The prevalence of fungi in drinking water and drinking water distribution systems and sources is well acknowledged. This study outlines the occurrence, monitoring, analytics of fungi in drinking water sources and distribution systems. The fungal diversity, mycotoxin occurrence and health implications in drinking water is also discussed. As a measure of microbial quality of drinking water, indicator bacteria are widely acknowledged and applied as standard parameters for compliance. However, bacteria and fungi are biologically different as microorganisms, with fungi being eukaryotes, it has been found that they tend to resist water treatment processes that are effective for bacteria. Water treatment processes such as filtration, ozonation, UV radiation, chlorination and chloroamination are commonly applied for microbial destruction in water including fungi. Available information from different studies indicate that fungi and fungal spores are more resistant than bacteria to the conventional water treatment methods thus bringing up the question of the sufficiency of use of set bacterial water quality standards as the main acceptable measure for all other microorganisms that have no set standards. Drinking water that is bacteriologically safe is not necessarily safe with respect to fungal contamination and thus there is need to move towards fungal specific water quality standards.

**P 051      Evaluation of hollow-fiber ultrafiltration for concentration of multiple pathogens from surface water**

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Hollow-fiber ultrafiltration for simultaneous pathogens concentration from surface water was compared with methods to concentrate virus and protozoans separately. Recoveries percentages were evaluated for Adenovirus (AdV), Rotavirus (RV), bacteriophage PP7, *Giardia*, *Cryptosporidium*, and *Escherichia coli*. The microbes were concentrated using dialyzers (Fresenius Hdf100S) with dead-end (DEUF) and tangential flows ultrafiltration. The primary concentrated was centrifuged and the pellet was used for quantification of protozoans with immunomagnetic separation and immunofluorescence assay microscopy; while the supernatant was submitted to secondary concentration with PEG-NaCl precipitation, acid nucleic extraction with QIAamp Maxi Extraction kit and Taqman qPCR or RT-qPCR for viruses quantification. Ultrafiltration methods were compared with Envirocheck filtration (USEPA Method 1623.1) for the protozoans and skimmed-milk flocculation method for virus concentration. All tests ( $n = 9$ ) were performed with 10 liters of surface water of rivers collected from three different sites. Overall, tangential ultrafiltration outperformed other methods with highest averages recoveries for all viruses evaluated. The values observed for tangential ultrafiltration, DEUF and skimmed-milk flocculation were respectively: 74%, 64.8%, 35.8% (AdV), 63.2%, 51.8%, 21.7% (RV), and 49.4%, 45.7%, 9.2% (PP7). Protozoal recoveries were higher in Envirocheck filtration compared with ultrafiltration systems. The averages recoveries for Envirocheck, tangential ultrafiltration and DEUF were respectively 65.6%, 54.10% and 52.3% for *Giardia* and 55.6%, 45.3% and 41.9% for *Cryptosporidium*. Average recoveries of *E. coli* were 66.2% for tangential ultrafiltration and 60.6% for DEUF. This study demonstrated that tangential ultrafiltration is more effective at recovering multiple pathogens from surface water when compared with DEUF and represents a low-cost method concentration for *Giardia* and *Cryptosporidium*, which can therefore be a useful tool for the investigation of environmental pathogens.

**P 052      WaterWiSe - a physicochemical test-bed for real time assessment of a tropical drinking water distribution system**

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Singapore Centre for Life Science Engineering

Engineered aquatic systems like those for drinking water distribution are always at risk of colonization by innocuous microorganisms that, even though inherently non-pathogenic, might act as pioneer species to form biofilms. Biofilms have been identified as environmental niches for the enrichment and growth opportunistic pathogens such as nontuberculous mycobacteria or *Legionella pneumophila* (Ashbolt 2015). In a tropical climate, increased temperatures and elevated precipitation rates can further increase the likelihood of biofilm growth, especially when monochloramine is utilized which has been described to provide a substrate to microorganisms and promote nitrification (Proctor and Hammes 2015).

The WaterWiSe long-term water quality assessment test-bed was created to monitor physicochemical parameters in a small scale drinking water distribution system (DWDS). Since 2016, seven nodes of water quality sensors were implemented in the operational DWDS of two campuses in Singapore (S and T) and have been utilized to measure leak- parameters such as pressure, flowrate, acoustics and water quality parameters like pH, ORP, conductivity, turbidity and temperature. Logged values have been transferred wirelessly to maintenance departments of the respective campuses and allowed for close to real-time response in the case of irregularities (malfunctions or accidents).

Measurements obtained by the WaterWiSe system enable in-depth analysis of, e.g. , the water age in the DWDS, to identify areas of stagnation, the prediction of water demands under regular (school term) and low-demand (vacation term) conditions as well as the demand of residual monochloramine. As those factors have been described to influence the growth of biofilm (Falkinham, Pruden et al. 2015), both mature and young swab samples have been analyzed using next-generation. Knowledge about microbial communities in DWDS can help estimate the risk associated with opportunist drinking water pathogens due to natural processes like heavy weather or outgrowth or accidents and contamination with surface water or sewage.

#### **P 053      *Legionella* in tourist facilities water system of a south Italy province**

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Epidemiological data show an increasing trend in legionellosis cases associated with travel, in fact the water distribution systems of tourist accommodation facilities are extremely sensitive to colonization due to their large size, to the complexity and to the seasonal use which determines long periods of standstill and stagnant flow. In this work, *Legionella* contamination levels of tourist accommodation facilities in a south Italy province (Taranto, Puglia) were evaluated, as prescribed by the environmental monitoring and control program carried out by the Regional Environmental Agency. In the 2015-2016 and 2016-2017 seasons, 45 tourist-accommodation facilities were monitored (22 in the first season and 23 in the second), for a total of 324 water samples, 136 and 188 respectively, taken from bathrooms taps and showers. The presence and concentration of *Legionella* was evaluated according to ISO standard n.1173:1998. Overall, 47.8% of the samples were positive; the percentage increased form 39.7% of the first season to 53.7% of the second. In both campaigns, more than 60% of the positive samples were detected by medium-sized facilities, with a number of rooms between 11 and 50. *Legionella pneumophila serogroup 1* was the most isolated strain in the 2015-2016 season, while *Legionella pneumophila serogroups 2- 15* in the subsequent. The majority (48%) of the positive samples in 2015-2016 showed a microbial count with an average of 373 CFU/L, while in the second season the more frequently positives samples (45%) resulted with higher values (average: 3908 CFU / L). The data showed a constant and increasing quantitative contamination, confirming a potential risk of infection for the travellers and the influence of facility size. The improvement of preventive measures to control the contamination and consequently protect health results important.

#### **P 054      Detecting *Legionella pneumophila* in Dutch water samples: comparing the national reference method with Legiolert**

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*Legionella pneumophila* is the causative agent of the human respiratory disease legionellosis. Nevertheless, the European legislation still have regulations focusing on *Legionella* species. The methods of detecting *Legionella* in water are based upon culture which is vulnerable to growth of other bacteria, therefore potentially underestimation of *L. pneumophila*. In this study, we quantitatively compared potable and non-potable samples in the Netherlands with the NEN 6265 (comparable to the ISO 11731) and the Legiolert system.

Each sample was analyzed using Legiolert according to the manufacturer's instructions and by NEN

6265. For NEN 6265, 250 ml of the sample was concentrated using membrane filtration, subjected to sonication, diluted again in 5 ml sample, mixed and heated at 50°C. A 0.1 ml aliquot was plated onto both BCYE and MWY agar plates. Isolates from the plates were confirmed as being *Legionella* by using MALDI-TOF. All positive results from Legiolert were also confirmed with MALDI-TOF. A total of 68 potable samples were analyzed and, of those, 16 (24%) were positive for presence of *L. pneumophila* using both methods. The NEN 6265 method identified 3 (4.4%) samples as *L. pneumophila* positive, which were not detected using Legiolert. Conversely, 4 (5.9%) of the samples were positive only by Legiolert. For the non-potable samples, a total of 69 samples were analyzed and, of those, 26 (38%) were positive for presence of *L. pneumophila* using both methods. The NEN 6265 method identified 2 (2.9%) samples as *L. pneumophila*, which were not detected using Legiolert. Conversely, 7 (10.1%) of the samples were positive only by Legiolert. In the 11 samples positive only by Legiolert, the NEN 6265 method failed to identify any *L. pneumophila*. Altogether, the new Legiolert method performed as well as the standard NEN 6265 method in detection of *L. pneumophila* in potable and non-potable water samples.

**P 055      Environmental surveillance of poliovirus: evaluation of concentration methods in wastewater**

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Environmental surveillance of poliovirus is critical to the endgame of poliovirus eradication, working as a supplementary action to acute flaccid paralysis surveillance with the identification of poliovirus circulation in endemic areas, detection of new poliovirus importations or new emergence of vaccine-derived poliovirus, as well as ensuring the elimination of vaccine strains. This study evaluated five methods of concentration of poliovirus from wastewater. Grab sampling was performed with four methods: PEG-dextran precipitation, method recommended by WHO, PEG-NaCl precipitation, beef-extract-celite filtration, and beef-extract organic flocculation. Trap sampling was also evaluated using gauze pad collected after three days submersed in sewage stream, following by elution and concentration with beef-extract flocculation. Recovery efficiency of all methods associated with grab sampling were evaluated by experimental contamination with Sabin-type-1 poliovirus (PV1). All concentrates were submitted to Sabin-PV1 detection by RT-qPCR and by inoculation in cell culture (5 L20B and 1 RD flasks to spiked concentrates; and 2 L20B and 1 RD to unspiked concentrates) following by PCR for Sabin-PV1 confirmation of flasks with cytopathic effect, according to the protocol of validation of concentration step (WHO Guidelines for environmental surveillance of poliovirus circulation, 2003). The averages recoveries by RT-qPCR were 30.7% (PEG-NaCl), 26.17% (beef-extract), 25.2% (celite), and 22.14% (WHO). PEG-NaCl technique was able to detect the highest number of positive unspiked samples (75%), following by OMS technique (50%). Only WHO and PEG-NaCl methods presented positive unspiked samples to Sabin-PV1 after cell culture-PCR tests. The percentages of Sabin-PV1 positive flasks inoculated with spiked concentrated (1:100 dilution) samples were 47.6% (PEG-NaCl), 35.7% (beef-extract), 21.4% (WHO), and 11.9% (celite). The PEG-NaCl concentration presented greater sensitivity to poliovirus detection in wastewater when compared with the other techniques evaluated. The results also confirmed that trap sampling with gauze pad is less effective than grab sampling to poliovirus isolation from environmental monitoring.

**P 056      Evalution of LegiolertTM for the detection of *Legionella pneumophila* and comparison with spread-plate culture and qPCR methods**

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*Legionella pneumophila* (Lp) is an opportunistic pathogen of great concern worldwide as a result of the large number of outbreaks. Spread-plate culture according to ISO 11731:2017 is the historical standard for detection and quantification of this organism, but detection and quantification of Lp by real time quantitative PCR (qPCR) is also widely employed. A novel alternative liquid culture method was developed based on the Most Probable Number (MPN) for the determination of Lp. Legiolert is introduced in a blister pack format as a powdered reagent for the testing of 100 mL of potable water and 1 mL of nonpotable water. Legiolert employs a selective formulation developed by IDEXX for the detection of Lp following incubation during 7 days with humidity. In the present study, we report the results of a comparison between ISO 11731, qPCR and Legiolert for the detection and quantification of Lp in potable and non-potable water samples.

Samples from non-potable origin (cooling towers, and air conditioning units) and potable water (cold and hot tap water, shower water, and thermal waters) from different origins (hospitals, industry, domestic, among others) and from different geographical locations within Portugal were analysed between September 2017 and February 2018.

Legiolert presented the highest percentage of positive samples for Lp (22%) followed by qPCR (17%) and ISO 11731 (6%). Enumeration with Legiolert ranged from <1 to 4.28log MPN L-1 (mean 3.42log), from 0 to 5.38log GC L-1 for qPCR (mean 4.03log) and from 0 to 2.81log CFU L-1 (mean 2.34log). The paired results between the different techniques varied between 78 % for ISO 11731 being Legiolert more sensitive and 90% for qPCR vs ISO 11731. Only 28% of the samples analysed were positive for the three methods. In conclusion, Legiolert is a valid alternative to the commonly used methods for the detection of Lp.

**P 057      Evaluation of flowcytometry and advanced live/dead discrimination**

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Generally, water quality is determined by the detection of fecal indicators using culture-based methods. Anyhow, culture based methods are time consuming. Flow cytometry allows the quantification of bacteria in water samples (total cell count) within minutes using a laser based microfluidic detection system. Fluorescent dyes allow labelling specific compounds of bacteria like nucleic acids. This can help to characterize water samples and indicate changes in the water matrix by contamination, stagnation or regrowth events.

Even though flowcytometry cannot substitute the detection of fecal indicators of specific pathogens in a water samples it is increasingly accepted and used at water suppliers for the control of water resources. Therefore, well-defined and adaptable protocols are required, valid for a variety of available flow cytometers. In this study, staining protocols for total cell counts, percentage of high nucleic acid bacteria and living cells in drinking, ground, and mineral water were compared and standard protocols evaluated. With standardized protocols and gating, round robin test results of four independent laboratories showed little deviation below 5 - 10 % depending on the water type.

In addition to total cell counts, fluorescent dyes allow viability monitoring. The red fluorescent dye propidium iodide exclusively labels membrane-damaged cells and allows a simple live/dead differentiation after heat treatment or chlorination. Anyhow, this principle does not work when membranes are unaffected by a disinfectant, e.g. UV treatment. Alternative dyes can be used to show cell viability, e.g. via protein activity or membrane potential. In this study, five different fluorescent markers were compared and proved to be suitable for live/dead discrimination after heat treatment, but showed limited effects for UV inactivation.

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**P 058      Use of Cloud Computing and Database Management for Conducting Environmental Surveillance of Poliovirus**

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Poliovirus is a human enterovirus that infects the nervous system and can cause paralysis. Since the World Health Assembly resolved to eradicate poliovirus in 1988, only three polio-endemic countries remain: Afghanistan, Pakistan, and Nigeria. As clinical cases have decreased, environmental surveillance has become increasingly important to monitor silent circulation of poliovirus within a population. However, disease tracking can be hindered by collection at incorrect or improperly-reported locations. Longitudinal data from inconsistent sites can confound verification of elimination. To address this issue, a novel web-based system called the Electronic Environmental Surveillance System (EESS) with a corresponding geolocation-tracking mobile application was developed to engage and track users throughout collection and concentration processes. EESS applies database management and cloud computing techniques to a novel sampling and concentration system, the bag-mediated filtration system (BMFS). Using EESS, Quick Response (QR) codes associated with samples are scanned throughout the process, automatically recording latitude and longitude and enabling chain-of-custody information to be recorded in the application. EESS allows for location, time and other data to be accessed in real time and managed online. EESS initial evaluation was completed by 15 volunteers. Each volunteer used the application at two different locations around the greater Seattle area. They scanned the provided QR codes and entered chain-of-custody information. GPS devices were provided, and the latitude and longitude of each location were recorded by the volunteers for comparison with values automatically recorded by the application. Out of 1,200 individual entries, 99% accuracy was observed in data transfer, and 100% accuracy in geolocation tracking. The low percentage of error indicates that environmental surveillance personnel can trust the system to provide accurate information. With modification, this tool could be applied to multiple sampling and collection methods. The improved database management provided could help to more effectively advance the global program for poliovirus eradication.

## **P 059 German-Australian Cooperation for Water Microbiology**

Claudia Stange, Jatinder Sidhu, Anne Roiko, Declan Page, Johannes Ho, Simon Toze and Andreas Tiehm

DVGW-Technologiezentrum Wasser, CSIRO Oceans and Atmosphere, Griffith University, CSIRO Land and Water, DVGW-Technologiezentrum Wasser, CSIRO Land and Water, DVGW-Technologiezentrum Wasser

Prompt water monitoring and quality control are essential for public health protection and risk regulation. Consequently, the rapid, specific and sensitive detection of microbial indicators and waterborne pathogens represent a key challenge in modern water quality monitoring. The overarching objective of the German-Australian collaboration ([www.gabi-cooperation.org](http://www.gabi-cooperation.org)) is to develop fast monitoring methods of microbiological key parameters.

The following research questions are main focus of both partners:

- PCR-based detection of hygienically relevant microorganisms in wastewater, surface water and groundwater
- Microbial Source Tracking using culture-independent methods
- Detection of clinically-relevant antimicrobial resistance genes #
- Improved methodology for the concentration and detection of viruses
- PCR-based live-dead differentiation
- Application of microarray technology in water monitoring
- Detection of pollutant degrading bacteria
- Nitrogen transformation

Initial work within GAbi focused on the detection of antimicrobial resistant bacteria, antimicrobial resistant genes and mobile genetic elements in different water matrices, like water used for drinking water production and bathing waters. The culture-based methods and molecular biological techniques for the detection of antibiotic resistance in the environment were successfully exchanged and applied to different studies. The next phase of work will evaluate the suitability of these methods for quantitative microbial risk assessment (QMRA).

Overall, the methods for molecular biological monitoring are being developed further to facilitate their use in routine diagnostics. For rapid exchange of data, references and methods, the "Joint PCR Reference Lab" will be established thus strengthening the German-Australian cooperation.

The authors gratefully acknowledge funding by the Federal Ministry of Education and Research (grant number 01DR17014).

## **P 060 What can flow cytometry tell us about drinking water quality?**

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In potable water analysis there is a need for rapid calculation of total bacteria present in treated water throughout the distribution network. Flow cytometry (FC) is increasingly applied for this purpose. Standardised protocols have been devised both in the literature and in the water industry, using DNA binding stains. However, the public health significance of FC counts is still unknown, and the relationship of this data to traditional water quality indicators such as coliform counts has not been well characterised.

Water chemistry is known to impact staining, but is currently not addressed in practical application.

Further, the diversity of environmental bacteria present in drinking water has been found to be surprisingly broad, and differential cell morphology and metabolism can hinder uniform stain binding over populations. Though these issues may reduce accuracy of flow cytometry counts, utilisation of multi-parametric data gained during analysis may allow different generalised groups of bacteria to be characterised. Working from this, water quality metrics could be devised and applied to network points such as service reservoirs, to give a baseline level of risk that is not reliant on count numbers alone. This can then be used to inform asset management programmes. Preliminary findings suggest use of DNA fluorescence emission profiles can be used to identify points which are prone to coliform failures, where this could not be shown using counts alone. Further, laboratory studies of environmental bacteria show that binding of SYBR green I is more reliant on cell biology than previously indicated.

## POSTER SESSION TUESDAY

### P 061 Microbiological assessment of fecal pollution in environmental waters impacted by Hurricane Harvey

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Hurricane Harvey originated as a tropical storm on August 17, 2017 over the Atlantic Ocean, becoming a Category 4 hurricane on August 25 as it approached the coast of Texas. Hurricane Harvey has caused unprecedented damages to huge parts of southeastern Texas, particularly damaging the wastewater infrastructure resulting in release of sewage contamination into environmental waters. The purpose of this study was to conduct a baseline assessment of fecal indicator bacteria (*E. coli* and enterococci) including human-associated fecal genetic markers (Human-specific *Bacteroidales*) across a Texas river impacted by Hurricane Harvey. We applied a suite of quantitative polymerase chain reaction (qPCR) assays targeting *E. coli*, enterococci, and human-specific *Bacteroidales* markers (HF183 and BacHum) to identify and quantify the fecal contamination in water. Water samples were collected along the Guadalupe River during September – December 2017. The most heavily flooded sites showed highest abundance of fecal indicator bacteria and human-specific *Bacteroidales* indicating that the large number of sewage overflows and stormwater runoff occurred during Harvey flooding introduced high levels of fecal bacteria into waterways draining into the Gulf of Mexico and impaired surface water quality. The human-specific *Bacteroidales* markers exhibited low to moderate correlation with conventional fecal indicators using qPCR results, suggesting the variable persistence of different markers and uncertainty of enterococci and *E. coli* for detection of human fecal pollution. In general, results of this initial microbiological contaminant assessment will serve as baseline information for ongoing follow-on studies to monitor existing and emerging public health risks to residents of Texas and potential long-term environmental impacts upon the water resources in the impacted regions.

### P 062 Peracetic acid as an alternative disinfectant for chlorine-resistant microbes such as *Clostridium perfringens* spores in chlorinated effluent from sewage treatment plants

(1) Hiroyuki Suzuki, (2) Kenji Oonaka and (1) Atsushi Hashimoto

(1) Pref. Univ. of Hiroshima, (2) Azabu Univ.

The enterotoxin gene (*cpe*) positive *Clostridium perfringens* and its spores, which cause food poisoning in humans, are ubiquitously detected in sewage effluent even after chlorination. Such effluents are considered to be an important pollution source of *C. perfringens* to surface waters such as rivers, which are used as domestic water and irrigation water sources.

In this study, we evaluated the effects of PAA against chlorine-resistant microorganisms. Although, PAA is used as a reducing the impact of treated-chlorinated sewage effluents on ecosystems or as a food disinfectant, the effect of chlorine resistant microbes in sewage such as *C. perfringens* is not clear.

After a 15-min reaction with sewage effluents and a single disinfectant, the free chlorine was reduced from 20% to 50%, whereas 80% of PAA remained (following previous studies, the initial concentration was 10 ppm for both). The CT values required for the 1.5-log inactivation of *C. perfringens* spores are 400 mg·min/L for chlorine and 800 mg·min/L for PAA. Even though both disinfectants exhibited inactivation effects proportional to the CT value, PAA showed a moderate effect compared to chlorine.

In 120-min exposure tests, an inactivation rate of 2.48 log for chlorine and 1.30 log for PAA was observed using each disinfectant alone. However, in an experiment using both disinfectants, the inactivation rate was 2.7 log, which was less than the additive value of the inactivation rates of the two individual disinfectants. This result indicates PAA and chlorine compete with each other. Therefore, under coexistence of chlorine and PAA, it is necessary to specify the method and order of the reaction.

Even though the inactivation effect is reduced in the presence of chlorine, these results show PAA can be applied to chlorine-resistant microorganisms such as *C. perfringens* as an effective disinfectant with a different mechanism of disinfection.

## P 063 Hospital Dialysis Water: Findings from a proficiency testing provider

Margaret Njenga and Nita Patel

Public Health England

### Introduction

The quality of water and associated dialysis solutions have been implicated in adverse patient outcomes. Haemodialysis patients are particularly vulnerable to contaminants in dialysis water. Therefore, it is important to have good quality water for patient safety.

The Food and Environmental Proficiency Testing Unit based at Public Health England provides External Quality Assessment to laboratories that analyses food and water samples. Hospital Dialysis Water Scheme (HDWS) is designed for laboratories that examine water used to prepare dialysis fluids in accordance with testing recommendations stipulated in published international methods. Dialysis water must comply with the minimum standard and shall contain <100 colony forming units per mL.

### Method

Three distributions per year are available; each contain two samples of simulated dialysis water. Data is collected on the methods/guidelines used, enumeration results and the interpretation of results. Interpretation of results is based on ISO:23500-5:2019 (Quality of dialysis fluid for haemodialysis and related therapies).

### Results

Participants results from 2013-2018 were compared worldwide for the HDWS. A range of methods and media are used to examine dialysis waters. Membrane filtration and Tryptone Glucose Extract Agar media was most commonly used. On average, 85% of participants reported an enumeration result within our statically calculated expected range. For interpretation of results, 80% of participants gave the correct interpretation. However, when results are borderline (50% of the maximum allowable colony count level), only 35% of the laboratories reported correct conclusion. This is due to some laboratories setting their own maximum allowable colony count levels. Although different media and processes have been recommended in ISO:23500-5:2019, laboratories should assess their selected method based on local validation criteria.

## **Conclusion**

EQA schemes is a valuable tool for microbiology laboratories in ensuring quality assurance of haemodialysis water. Participation in PT identify gaps in processes by highlighting where quality improvements can be made.

### **P 064      Detection of non-tuberculous mycobacteria in endoscope rinse waters: findings from an external quality assessment provider.**

Zak Prior and Nita Patel

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#### **Introduction:**

Non-tuberculous mycobacteria (NTM) are emerging pathogens in immunocompromised patients. There are over 170 different species with varying ability to cause diseases of differing severity. Water systems are primary sources of infection; therefore, it is imperative that regular water testing for NTM is a well-established practice.

Culture based detection of NTM remain the method of choice, albeit time consuming due to long term incubation, potentially delaying therapy and subject to overgrowth from microflora and misdiagnosis. Identification to species level by reference laboratory confirmation is important for species dependent antimicrobial susceptibility and patient care.

The Food and Environmental Proficiency Testing Unit (FEPTU) based at Public Health England provides international microbiology external quality assessment (EQA) schemes to laboratories that analyse NTM in endoscope rinse waters.

#### **Methods:**

A range of *Mycobacterium* species, colony forming units per 100 mL and background flora were used in the simulated samples. Data was collected on the methods/guidelines used, detection and enumeration results. Worldwide participant's results were qualitatively analysed for NTM detection in six positive and two negative samples.

#### **Results & Discussion:**

Participants achieved a high percentage of correct results (range 92-100%) for identifying negative samples, however the correct detection in positive samples was more varied (range 61-88%).

Variation in methods, incubation temperature and time, media, and equipment; as well as strain specific phenotypic characteristics and interfering background organisms; and non-accredited participants using the scheme for method validation, all contributed to the varied success of detection.

#### **Conclusion:**

EQA supports the quality assurance of NTM water testing in laboratories by highlighting issues with test and method performance. It assists in the validation and improvement in methods used by highlighting variation and inconsistencies in results so quality improvements can be made. This ensures that surveillance data can be compared both nationally and internationally.

**P 065      Bacterial community dynamics of biofilms in two university campus drinking water distribution networks**

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The development of biofilms in drinking water distribution systems (DWDSs) can affect both water quality and distribution system integrity. To understand the factors influencing microbial communities of biofilms within an operational DWDS, two campus testbeds (S and T) mimicking the city water distribution system in Singapore were created in this study.

Mature biofilms were collected from the wall of the pipes during routine maintenance and replacement operations and young biofilms from the water quality sensors which had been embedded in the campus DWDS testbeds with online measurements of physiochemical water quality parameters. Bacterial communities of biofilms were analyzed using 16S rRNA amplicon sequencing targeting V3 to V6 region. The composition of families with higher relative abundance in the biofilms of mature pipes tended to resemble those in young biofilms at the same campus testbeds, like *Mycobacteriaceae*, *Methylobacteriaceae*, *Rhodospirillaceae*, *Nitrosomonadaceae*, and *Moraxellaceae*, but not *Pseudomonadaceae*.

Further analysis identified a core bacterial community among all young and mature biofilms in both campus testbeds. A change in conductivity shaped the nascent communities of both young and mature biofilms regardless of testbed location, which may be associated with a change in the source water. Shifts in community composition from Firmicutes to Alphaproteobacteria could be observed for the young biofilms in early developmental stages.

This study demonstrates the effect of operational changes on the microbial community of biofilms within an operational network. It also enhances our understanding on the dynamic succession process of biofilms within DWDS.

**P 066      Analysis of bacteriological quality of domestic water sources in Kabale municipality, Western Uganda**

Alex Saturday

Kabale University

**Background:** Worldwide, water is an important component of living beings as it performs unique and indispensable activities. However, due to many anthropocentric activities, freshwater resources are getting deteriorated at a faster rate. In Africa, estimates indicate that 327 million people without access to safe drinking water live in Sub-Saharan Africa (WHO & UNICEF, 2014). Absurdly, people in Tanzania, Kenya, and Uganda still drink unsafe water in spite of improved access to safe drinking water. This study, therefore, aims at assessing bacteriological quality of drinking water sources in urban areas, a case study of Kabale Municipality, Southwestern Uganda.

**Material and Methods:** Water samples from water springs were collected aseptically in sterilized 500 ml sterile plastic bottles and them transported to the laboratory for analysis. Membrane filtration method was used for analysis of water samples in accordance with APHA, (2005).

**Results:** The mean *E.coli* concentrations, total coliforms, and THB were 24.07CFU/100 ml, 85.71 CFU/100ml, and 197.07 CFU/100 ml, respectively in the wet season. While in the dry season, the mean concentrations were 2 CFU/100 ml, 10 CFU/100 ml, and 91 CFU/100 ml for *E.coli*, total coliforms, and THB, respectively. There were significant differences between CFU of total coliforms, HTB, between wet and dry seasons ( $p = 0.026$ ). The concentration of bacteria in water sources did not conform to WHO drinking water quality guideline value of no detection per 100 ml.

**Conclusion:** With the present state of knowledge from this study we can conclude that; majority spring water sources are located in less than 20 meters away from residential areas with significant paved areas, the presence of septic tanks and pit latrines; wet season significantly affects the quality of domestic water sources than dry season; majority drinking water sources were in excess of WHO recommended guidelines for drinking water in wet season.

**P 067      The value of proficiency testing for health related water microbiology – why is it useful to you?**

Nita Patel

Food and Environmental Proficiency testing Unit - Public Health England

The Food and Environmental Proficiency Testing Unit (FEPTU) provides international microbiology proficiency testing (PT) schemes to laboratories that analyse food, water and environmental samples. Laboratories that examine healthcare related water samples need to produce results that are reliable, accurate and clear. In addition the correct conclusion needs to be provided so that essential equipment can be appropriately managed if found to be contaminated. This ensures that the public is protected from harm. We in FEPTU understand what the potential public health impact can be by using unsafe water.

Hospitals must ensure that water used in their healthcare facilities, is safe to minimise the potential risk of infection to people receiving treatment within the hospital environment. However in recent years published evidence has revealed, an increase in outbreaks and incidents of infection where the microbial source can be traced to contamination of the water used in hospitals.

FEPTU provides five accredited hospital water PT schemes; Endoscope Rinse, Dialysis, Hospital Tap, *Legionella* Isolation and Hydrotherapy Pool.

The presentation will give an overview of the schemes, the purpose and what the objectives are. Some of the schemes allow participants to report a conclusion based on their test results and guidelines followed. The PT results to date show that participant still provide an incorrect conclusion; this can lead to essential hospital equipment being managed inappropriately with the potential to cause harm. Performance in PT will be presented along with the educational value gained.

Participation in PT helps to identify gaps in laboratory processes, highlighting where quality improvements can be made. It provides an opportunity to improve staffs' knowledge and experience with organisms not frequently encountered. Regular participation provides performance data that helps to demonstrate compliance with testing standards, thereby meeting and maintaining accreditation requirements.

**P 068      Drinking-water Quality in Healthcare Centers in Costa Rica during 2017**

Darner Mora-Alvarado, Pablo-César Rivera-Navarro

Costa Rican National Water Laboratory (Costa Rican Water and Sewage Institute; Instituto Costarricense de Acueductos y Alcantarillados AyA).

This study aims to assess drinking-water coverage within healthcare centers in Costa Rica during 2017. The main objective is to establish the baseline in order to define national targets for 2022 and 2030 within the scope of the Sustainable Development Goal 6: Clean Water and Sanitation.

The methodology consisted of describing social security in healthcare centers, assessing drinking-water supplied at healthcare centers according to Costa Rican regulation (microbiological parameters i. e. fecal coliforms, *Escherichia coli*, *Pseudomonas aeruginosa* and heterotrophic plate count), and applying the WHO/UNICEF Joint Monitoring Program (JMP) ladder for drinking-water using water sources and water supply network samplings.

Results show that 97 % of the water sources and 87.5 % of the supply network provided clean drinking-water in compliance with Costa Rican regulation. According to the JMP ladder, coverage of drinking-water supply network in healthcare centers are the followings: 87,5 % is qualified as safely managed service, 12,5 % as basic service, 0 % as limited service, and 0 % as unimproved service. However, when assessing source water coverage in healthcare centers the results show 97 % is qualified as safely managed service, 3 % as basic service and 0 % as limited or unimproved services. The national targets for drinking-water supply network coverage in healthcare centers are 95 % coverage for 2022 and 100 % for 2030. Whereas, the national target for source water coverage are 99 % for 2022 and 100 % for 2030.

This study strongly recommends Costa Rican healthcare centers to participate in the social environmental programs such as *Bandera Azul Ecológica* and *Sello de Calidad Sanitaria*. These programs encourage healthcare centers to improve their drinking-water quality control.

**P 069      Drinking water and faucet surface monitoring related to a *Pseudomonas aeruginosa* outbreak at a pediatric hospital in San José, Costa Rica**

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Hospital drinking water and faucet surfaces are potential sources of healthcare-associated infection (HAI) microorganisms. Costa Rican drinking water standards for healthcare centers dictate the absence of fecal coliforms, *Escherichia coli*, and *Pseudomonas aeruginosa*, as well as less than 30 CFU/ml heterotrophic plate count (HPC). Despite drinking water quality monitoring and HAI surveillance protocols, a *P. aeruginosa* outbreak occurred at the Costa Rican National Social Security Children's Hospital during 25 days in 2017. In an attempt to explore the source of the outbreak, the drinking water supply source water, storage tank, and supply network as well as faucet surfaces were evaluated for the aforementioned bacteria per Costa Rican standards using standard culture-based techniques and antimicrobial susceptibility testing (Vitek 2®). Quantitative PCR (TIB MolBiol®) for detecting *Legionella pneumophila* was also included to detect other HAI-related microorganisms. *P. aeruginosa* was identified in two of six water samples and 8 of 22 faucet surfaces. No samples were positive for *L. pneumophila*, *E. coli*, fecal coliforms, and HPC met drinking water standards. Interestingly, antimicrobial susceptibility tests exhibited variability among *P. aeruginosa* strains isolated from water and faucets. Furthermore, those *P. aeruginosa* strains isolated from water and faucets had antimicrobial susceptibility patterns that differed from those found in the affected patients. Consequently, no associations between *P. aeruginosa* isolated from the drinking water network nor faucet surfaces were established. These results suggest that *P. aeruginosa* were transient colonizers in water and on faucet surfaces. While drinking water and faucet surfaces were not identified as sources involved in the *P. aeruginosa* outbreak, it is possible that these associations were not observed due to the number of samples analyzed. Future studies are needed to fully understand the role of hospital drinking water and faucet surfaces in the transmission of HAIs.

**P 070      Occurrence of toxin-producing cyanobacteria and associated toxins in a mixed-use coastal catchment**

Hannah P. Sassi, Emily White, Kansas Keeton and Floris van Ogtrop

The University of Sydney

The Hawkesbury-Nepean Catchment (HNC) in New South Wales, Australia spans ~21,000 km, flowing west from the Pacific Ocean and into the Tasman Sea. It is a mixed-use, peri-urban catchment that supplies water for drinking, irrigation, recreation and grazing. In more saline portions, it is the second largest area of oyster and prawn production in the state. Due to the catchment's geographic location, salinity decreases as it flows inland (west). This catchment provides a unique environment

for cyanobacteria to bloom and then subsequently lay dormant. This may expose water users to toxic blooms from both saline and freshwater species. Due to the recent blooms in Australia and abroad, isolating toxin-producing cyanobacteria, and cyanotoxins has become necessary to accurately evaluate water quality for direct consumption and irrigation for food crops. Food is considered a major exposure route for cyanotoxins, especially fresh produce and seafood. Due to this, a sampling campaign is currently underway for the HNC which consists of 13 consecutive months of water and sediment sampling, including extensive, in-field physicochemical measurements. Samples are filtered, extracted and relative abundance and diversity is evaluated through 16S sequencing (MiSeq). DNA extracts from sediment and water will also be analyzed using a multiplex qPCR assay for toxin-producing genes from *Nodularia spumigena*, *Dolichospermum circinale*, *Microcystis aeruginosa*, and *Raphidiopsis raciborskii*. These organisms produce microcystin, nodularin, anatoxin-a, and cylindrospermopsin toxins. Physicochemical parameters are being evaluated to determine environmental conditions favorable for toxin-producing species, and cyanotoxin presence. Currently, the sampling campaign is half-way complete. Pilot data has shown that Cyanobacteria account for approximately 15-35% of community structure in our sites. Preliminary analyses of pilot metadata show high levels of DO (>100% saturation) and chlorophyll-a (>150 µg/L) correlated to the presence of cylindrospermopsin-producing genes in the water for a produce farm-adjacent site, as determined through conventional PCR.

## P 071 Proliferation of coliform bacteria in drinking water reservoirs, dams and lakes

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Due to their presence in the gut of humans and warm-blooded animals, coliform bacteria have been used as an indicator for fecal contamination in drinking water. Yet, in contrast to *E. coli*, coliform bacteria are not necessarily of fecal origin, as several genera are also found in natural environments and in association with plants and invertebrates. Furthermore, coliform bacteria can multiply in drinking water systems under certain conditions, like e.g. in thick biofilms or in sediments. As a consequence, coliform bacteria are presently seen as indicative of microbiological water quality deterioration.

Worldwide, surface water is a major source for drinking water production with dams as important raw water reservoirs, especially in regions with water scarcity. Since several years, mass proliferations of coliform bacteria have been observed in dams and lakes used for drinking water production in Germany, like e.g. Lake Constance. During the summer months, coliform bacteria can reach densities of up to 100.000 cfu/100 ml in these oligotrophic environments. These high densities of coliform bacteria in the raw water challenge the treatment of the waterworks, as they mostly use conventional treatment techniques (e.g. flocculation and filtration).

Our monitoring studies indicate that the proliferation of coliform bacteria is an autochthonic process within the water column, with only a few strains of coliform bacteria being capable of building up these “coliform blooms”. Some strains are members of the *Enterobacter* complex that also includes hygienic-relevant strains. Interestingly, very closely related strains are found during blooms in different reservoirs all over Germany.

Due to higher water temperatures, coliform blooms are expected to occur more frequently in the near future. Within the current project, we aim to analyze the reasons behind these mass proliferations of coliform bacteria. The genomes of these coliform bacteria are studied in order to reveal special adaptations for living in oligotrophic environments such as drinking water.

**P 072      Adaptation of waterborne echovirus to warm habitats enhances disinfection resistance**

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Because of their high mutation rate, enteric virus populations excreted from a host are not clonal, but rather consist of a mutant cloud with many different genetic variants. Among these variants, the susceptibility to environmental stressors (e.g., temperature, sunlight, microbial grazing) is expected to differ, and the variants most resistant to environmental stressors are the most likely to persist and encounter a new host. Interestingly, several studies have isolated viruses from the environment with a significantly higher resistance to disinfection compared to the corresponding lab strain. This indicates that environmental persistence and disinfection resistance may be linked.

The goal of this study was to determine if exposure to, and persistence under, environmental stress leads to virus resistance to disinfection. Using echovirus 11, we first conducted a series of experimental evolution experiments under different combinations of solar irradiance and temperature, representative of those encountered in temperate and tropical climates. We then determined the persistence of the adapted echovirus 11 populations with respect to thermal inactivation, solar inactivation and microbial grazing, as well as the susceptibility to inactivation by chlorine.

None of the adapted populations exhibited enhanced persistence to sunlight or microbial grazing. However, the populations adapted to high temperatures (30 °C) were less prone to thermal inactivation and exhibited a higher capsid stability compared to those adapted to lower temperatures. In addition, the 30 °C-adapted populations were also more resistant to inactivation by chlorine. Interestingly, the mutations associated with thermal stability are commonly found in circulating echovirus 11, indicating a widespread distribution of not only of high environmental persistence, but also disinfection resistance. Overall, our data indicate that viruses that persist in warm surface waters may have enhanced disinfection resistance. As such, disinfection requirements may differ between climate zones, and may change as a function of global warming.

**P 073      Fate of wastewater in the Environment - A synthesis for the WHO European Region**

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The WHO Global Health Risks report of 2009 identified unsafe water and sanitation as one of the leading risk factors affecting human health. Many microbial contaminants are discharged into the environment through wastewater treatment plants (WWTPs). Where WWTPs are absent, the disposal of human excreta is unsafe and concentrations of the microbial contaminants in the aquatic environment could be much higher. In the WHO European Region, 67 million people lack access to improved sanitation facilities and could thus be at risk. As part of the UNECE Protocol on Water and Health, a synthesis study was undertaken to identify the fate of wastewater in the environment of the WHO European Region.

A search of the EMBASE database was performed using search terms with key words such as 'sanitation' and 'water contamination', combined with (amongst others) 'microbial contamination' and 'environmental monitoring'. Contaminants with known impacts on the environment or on human health, as identified in the recently published WHO guidelines on sanitation and health, were included as search term in the query.

Of 1591 scientific articles, a total selection of 334 articles linked contamination to sanitation and were screened using text mining methods.

Twenty four percent of all articles mention research on microbial contaminants. Bacteria were most frequently studied (63%), with *Escherichia coli* as the main contaminant. Research on viruses (37%), protozoa (27%) and parasites (14%) was less common. Countries with the highest number of case studies were Spain, Italy, Germany and Finland. Surface water contamination was described in countries all over the WHO European Region, sometimes leading to outbreaks. Where innovative WWTP techniques were in place, the water quality of the effluent increased and human and environmental health issues decreased. This study identified the uneven distribution and the lack of information on sanitation across the Region. Future research should fill this gap.

**P 074 Physicochemical point of view in the evaluation of PR772 bacteriophage as a surrogate for human adenovirus**

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Among pathogenic enteric viruses, human adenoviruses (HAdV) are characterized by numerous structural specificities. Indeed, the diameter of their icosahedral capsid is about 90 nm with projecting fibre of 30 nm at each vertex and their genome is a linear double stranded DNA of 35-36 kbp, whereas other pathogenic enteric viruses are RNA viruses with an icosahedral capsid of about 25 nm without fibre. But HAdV are also noteworthy by their high frequency from surface water to drinking water which is explained by their faecal excretion whatever the induced symptoms and by their resistance to environmental conditions and to some disinfection treatments such as UV and monochloramines. These particularities raise the question of the behaviour of HAdV in water. Because of their structural, genomic and replicative similarities, tectiviruses such as bacteriophages PRD1 and PR772 have been proposed as potential surrogate for HAdV. Thus, bacteriophage PR772 appeared as a promising surrogate in the understanding of HAdV type 2 inactivation by free chlorine. The aim of the present work was to compare the physicochemical characteristics of HAdV type 2 and bacteriophage PR772 in order to evaluate this phage as a surrogate to help understand the behaviour of HAdV in water networks. The isoelectric charge of both particles was monitored in different pH and ionic strength conditions. The values of the isoelectric point (*pI*) of HAdV type 2 and bacteriophage PR772 were between 3 and 4, meaning that these particles are negatively charged at neutral pH. Adhesion experiments on hydrophobic polystyrene beads were performed to compare the hydrophobicity of both particles. Hydrophobicity appeared as the main difference between HAdV type 2 and bacteriophage PR772. The role of hydrophobicity in the accumulation of viruses on surfaces might limit the use of bacteriophage PR772 as surrogate of HAdV in such experiments.

**P 075 Fate of marine fish pathogen *Tenacibaculum maritimum* and fecal indicator organisms in sediment-water microcosms**

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With increasing global demand for seafood and fish, the aquatic food supply has transitioned from being primarily based on wild capture of fishes to aquaculture. *Tenacibaculum maritimum* is one of the most important fish pathogens in coastal marine waters fish farms. Uneaten feed and fish feces tend to aggregate and sink to the bottom of the sea; yet little is known about its impact on sediment-based microbial communities and the ability of pathogens to prevail in sediment-based biofilms. The aim of this work was to assess the fate and transport of *T. maritimum*, one bacterial and one viral

model organisms in sediment-water microcosms and to study the effect of macroaggregates on decay.

Outdoor microcosms containing water (200 ml) and sediments (100 g) were inoculated with *T. maritimum*, *Enterococcus faecalis* and bacteriophage P22. Triplicate microcosms were spiked with either planktonic cells/phages or with target organisms attached to macroaggregates. Separate controls with unspiked seawater and unspiked macroaggregates were also included. Decay in the aqueous and sediment phases was studied for 14 days. Cultivation-based methods and real-time PCR were used to analyze cultivable cells/particles and DNA levels, respectively. Physicochemical variables (pH, turbidity, dissolved oxygen, temperature, conductivity) were also measured. Decay constants and the time for a one log reduction (T90) were calculated employing a first order decay model. These constants will be used in a mass balance model during flume experiments.

Results showed that cells/particles decayed more rapidly than total DNA for all treatments and surrogates, as expected. P22 persisted the longest in both phases and remained infectious to the host organism *Salmonella typhimurium* during the experiment. Furthermore, target organisms in sediments showed no decay except for *T. maritimum* which had decay rate constant lower than those in water.

**P 076 Human pathogenic viruses and indicators in urban stormwater runoff in the San Francisco Bay area**

Katherine Graham and Alexandria Boehm

Stanford University

Stormwater runoff from urban environments sweeps microbial pollution from impervious surfaces to waterways. Bacteria, protozoa, and viruses, many of which are pathogens, have been detected using molecular biology and culture-based assays. Despite this, we have limited data on the concentrations and viability of pathogens in urban stormwater runoff. In particular, human pathogenic viruses, such as adenovirus, are important to fully characterize due to their low infectious dose and recalcitrance in the environment. Furthermore, since viruses in water are not usually directly quantified and we rely on indicators to infer their presence, it's important to compare these pathogens to commonly used indicators. Traditionally, fecal indicator bacteria (FIB) have suggested pathogens in various environments, but their accuracy is questionable. A new viral fecal indicator, crAssphage, is being investigated as an alternative to traditional methods.

This study aims to assess the following hypotheses: 1) Urban stormwater runoff in the Bay Area represents a source of enteric viral pathogens (norovirus GI and GII, adenovirus, and enterovirus) and indicators (FIB and crAssphage) to the SF Bay; and 2) enteric viral pathogen concentrations in this study are correlated with human fecal indicator concentrations.

We visited six urban creeks in the San Francisco Bay area biweekly from October 2018 to March 2019. We collected samples from each creek and quantified FIB using EPA methods. 10-L samples were concentrated and archived for later virus quantification. DNA and RNA were extracted from filters and qPCR assays were run for adenovirus and crAssphage; RT-qPCR assays were run for enterovirus, norovirus GI and GII. Additionally, viruses were extracted from filters and viability was determined using plaque assays for adenovirus and enterovirus. Using this approach, we can begin to assess the risk of viruses to human health in urban creeks in the SF Bay area and how well indicators capture this risk.

**P 077      Occurrence of *Naegleria fowleri* in private and public water systems in Louisiana**

Samendra Sherchan

Tulane University

*Naegleria fowleri* (*Nf*), also known as “the brain-eating amoeba”, is a pathogenic free-living amoeba found naturally in hot springs and warm surface waters. It can cause primary meningoencephalitis (PAM), most often leading to death within 4 to 6 days if it enters via the nasal cavity when swimming or diving. This amoeba is already listed in the United States Environmental Protection Agency (USEPA) draft Contaminant Candidate List 4 (CCL4). In 2011, two people died of PAM caused by *Nf* in DeSoto and St. Bernard Parishes in Louisiana. Both cases involved treated tap water that lacked detectable chlorine residual that was self-introduced into the sinus cavity via neti-pots. In 2013, there was a second death in St. Bernard Parish (4-year old boy) caused by PAM and confirmed to be *Nf* infection. The route of exposure was inhalation of treated tap water while the child played on a Slip n' Slide (water slide). To date, a total of nine Louisiana's public water systems have been tested positive for *Nf*. The USGS estimates that 13% of Louisiana population which equals to 588,000 people use groundwater from privately owned wells for their domestic water use. Domestic well water withdrawals in LA occur mainly in rural areas where public systems were not available. U. S. Environmental Protection Agency (EPA) regulates water from public sources, but it does not monitor private wells. This presentation will cover the results of our study investigating the occurrence of *Nf* in public and private water systems in Louisiana.

**P 078      Comparison of removal rates for *Bacillus subtilis* spores for different flow conditions**

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Subsurface media are being used around the world as a means to mitigate microbial contamination, but vary widely in their ability to remove microorganisms. The aim of the study was to provide the necessary knowledge for the retention of microbial contamination in groundwater and to receive reliable parameters for the calculation of safe setback distances for drinking water wells. We used tracer tests in the field and column tests in the laboratory to evaluate the ability of subsurface media to attenuate *Bacillus subtilis* spores.

In this study we used different tracers with a special focus on *Bacillus subtilis* spores as a microbiological tracer. These endospore-forming bacteria are of similar size as many important bacterial pathogens such as *Campylobacter* and *Salmonella* spp. However, the spores are much more persistent during transport and are therefore conservative tracers to study filtration of bacteria in the subsurface.

The novelty of this work is the comparison of removal rates for *B. subtilis* spores under three different flow conditions. These flow conditions were controlled by changing the extraction volume from a pumping well in an alluvial gravel aquifer near Vienna, Austria. The samples were taken from a pumping well at a distance of 25 m from the injection point. During the tests, the pumping rate was set to 1, 5 and 10 l/s, respectively. This provided us with insight to the characteristics of microbial transport at the study site, and how this changed with different pumping rates. It was found that an increase in pumping rate has a negative effect on the retention rate of *B. subtilis* spores during aquifer transport.

This is a joint investigation of the Interuniversity Cooperation Centre for Water & Health ([www.waterandhealth.at](http://www.waterandhealth.at)).

**P 079 Molecular Detection of Opportunistic Pathogens in Rural Louisiana's Drinking Water Distribution System**

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Tulane University

In the past decade, opportunistic pathogens such as Legionella spp., Naegleria fowleri, E. coli and Mycobacterium avium have been found in drinking water and have become a major public health concern. According to the CDC, opportunistic pathogens of premise plumbing (OPPPs) are waterborne microorganisms that emerge in drinking water distribution systems (DWDSs), and cause diseases in individuals with predisposing conditions, such as age (>70 years), cancer or immunodeficiency. Opportunistic premise plumbing pathogens in drinking water distribution systems are responsible for causing numerous infections through the consumption of drinking water. The number of opportunistic pathogens and the number of individuals at risk of contracting infections caused by opportunistic pathogens in drinking water has risen drastically in the past decade. Preflush and postflush water samples were collected from 64 houses in a town in northeast Louisiana to determine drinking water quality in terms of understanding abiotic and biotic factors on potential proliferation of OPPPs. Physical and chemical water quality parameters, such as pH, temperature, dissolved oxygen, salinity, and specific conductance were measured. These water sample underwent filtration, DNA extraction, and finally qPCR. The qPCR results indicated that Legionella was found in 72% of the samples and had the highest prevalence followed by Mycobacterium in 67% of the samples, E. coli in 48% of the samples and N. fowleri in 6%. The results indicate that thermal disinfection of water in drinking water distribution systems can reduce the persistence of Legionella and therefore reduce the risk of contracting Legionella spp. and other opportunistic pathogens.

**P 080 Challenges in provision of laboratory water quality testing facilities in Low- and Medium-Income Country (LMIC) settings**

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Water quality testing plays a key role in public health through the evaluation of water quality interventions and operational monitoring of drinking water supplies. The ambitious United Nations Sustainable Development Goals' target to achieve 'universal and equitable access to safe and affordable drinking water for all' by 2030 reinforces the need for water quality testing capabilities in low- and middle-income countries (LMIC). Testing is required not only to evaluate the quality of drinking water as supplied, but also to assess the sustainability and equity of safe water provision over time.

We report our experience in setting up a laboratory facility in a remote location in India. We selected the laboratory location based on the availability of an electricity supply metered for commercial purposes, mains water supply, mobile phone connectivity, proximity to study villages (maximum length of round trip to each approx. 3 hours), good road connections between villages and to commercial centres, and a nearby educational institution from which to recruit staff. Obstacles encountered were administrative (e.g. permit acquisition for biohazard waste disposal), staff recruitment and retention, power interruptions, procurement and transport of laboratory consumables, and provision and maintenance of bacterial reference cultures. Despite these, we implemented a water monitoring program comprising laboratory analysis of 5500 samples for faecal indicator bacteria and physico-chemical parameters using standardised procedures. We performed routine intra- and inter-laboratory checks of the reliability of results. We attribute our success to good staff training, attentive supervision through visitation and regular teleconferencing, good record keeping, and use of technology. Methods for microbial testing of water are continuing to evolve, with the likely eventual replacement of existing faecal indicator methods with molecular techniques targeting pathogens. Regardless, we believe the principles employed are relevant to planning and designing laboratory processes and facilities to perform complex analyses in LMIC settings.

**P 081 Differential response of microbiological indicators of water quality to diverse anthropogenic disturbances**

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Expanding population coupled with environmental degradation is an evolving problem in low developing nations. This expanding population extensively interacts with river water in the course of meeting their social and economic demands. However, the study regarding the response of microbial indicators of water quality to diverse human disturbances in the river water of developing nations is lacking.

As a result, this research unveils human disturbances in River Ngerengere in Tanzania and links these disturbances with concentrations of microbial indicators of water quality determined in the river water using standardized methods.

Of the disturbances identified, in-river washing (e.g. washing of motor vehicles), animal watering, agricultural irrigation, wastewater treatment effluent and river channelization significantly elevated concentration of *Clostridium perfringens* (CP) and *Escherichia coli* (EC) in the water column while reducing substantially the concentration of *Pleurocapsa* (PP) (belonging to Cyanobacteria). Channelization contributed significant number of CP (69%) than EC (31%) while wastewater effluent contributed more EC (75%) than CP (35%). In contrast, significant numbers of PP were detected in the drinking water extraction point compared to CP and EC. Drinking water extraction did not alter microbiological quality of investigated river water cause the values of microbial water quality indicators before and after drinking water extraction points were not significantly different ( $P>0.05$ ). Further, PP/CP ratio was higher in the drinking water extraction points than in the rest of the investigated sites.

To this end, this study showed that microbial indicators of water quality had contrasting response to anthropogenic disturbances in which PP were abundant in sampling points free from contamination while EC and CP were elevated in the sites disturbed by wastewater effluent and channelization, respectively. These findings are necessary for the development of successful water quality management strategies and to develop predictive models, which would aid in forecasting of riverine water quality.

**P 082 From waste to wow – Low cost green technology for domestic wastewater treatment for reuse and beneficiation**

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Council for Scientific and Industrial Research, Water Resources Management Centre

Phycoremediation makes use of macroalgae or microalgae and can be used to treat wastewater. This technique has the potential to be used as an alternative biomass source for bio-energy production. The current study utilizes a specific consortium of algal species (isolated and cultured in the laboratory) to reduce nutrients and create conditions suitable for effective reduction of pathogens in WWTW as well as reclamation of water in water scarce countries. The aim was firstly to implement a self-sustaining system that is independent of electricity or expensive chemicals and that can be effectively operated within the current financial and capacity constraints of developing countries using existing infrastructure i.e. waste stabilisation pond systems. Secondly, it was to establish the feasibility of algae biomass generated from maturation ponds as bio-energy. This low cost green technology has already been rolled out with great success at two waste water treatment works in South Africa. To date, total phosphate removal efficiencies of 87.1% and nitrogen levels of 56.3% was achieved in final effluent. *E.coli* numbers were reduced to below Department of water and sanitation guideline levels. Under auspices of the African Development Bank's Africa Climate Technology Centre, the research team recently started a small scale pilot plant for the drying of algae biomass under natural climate conditions at the rural Brandwacht domestic waste water treatment plant. The next step will be to determine the algal biomass that can be generated for bio-energy using a specific consortium of algae under natural drying conditions. If enough algae biomass is generated, the team will explore the possibility of using this algae biomass to generate bio-energy for the small rural town of Brandwacht. Pending a health risk assessment, bio-fertiliser production or pelleting the biomass for animal feed are other products under investigation.

**P 083 Development of Zeolite-Ag immobilized non-woven cloth indicating the antimicrobial activity in drinking water**

Chieko Shinohara, Naoto Matsue, and Hiroshi Hirotani

CS, HH: Osaka Kyoiku University, NM: Ehime University

In the developing countries, there still remains the population that lack access to the safe drinking water. To improve this situation, we developed an antimicrobial cloth, an un-woven fabric on which zeolite particles harboring Ag<sup>+</sup> were immobilized. The water to be used for drinking may be stored in the vessels with this piece of cloth in an each household, and during the storage, the water may be disinfected by the cloth and at the same time some positively charged heavy metals are expected to be adsorbed and removed by zeolite particles. We examined the production method of the cloth and tested the performance of disinfection by reduction of *Escherichia coli*.

A polypropylene-polyethylene un-woven fabric was cut into 5cm by 5cm pieces. Zeolite particles were sprinkled evenly over the cloth. The cloth was heated to promote immobilization of zeolite to the fibers, followed by shaking in water at 120 strokes/min for 1 h to remove zeolite particles that were not immobilized to the fibers. The fabric was placed in AgNO<sub>3</sub> solution to exchange Ag<sup>+</sup> with H<sup>+</sup> on the surface of zeolite particles for 2 h. The piece of cloth was air-dried and subjected to antimicrobial activity tests.

The pieces of the cloth were placed in sterilized tap water, in which *E. coli* was spiked, for a certain period of time. The effect of heating temperature and length used in the production of the cloth, on the antimicrobial activity was investigated.

To immobilize zeolite on to the fabric, the heat treatment at 160C for more than 20 min was needed.

The antibacterial activity was dependent of the amount of Ag+ attached to the zeolite. It took 2.9 min for the 99% reduction of *E. coli* when 56 µg Ag+/cm<sup>2</sup> was attached.

#### **P 084      Study on indicators of SDGs and support of improved access to safe water in UGANDA**

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WHO (2014) estimated more than 10 800 deaths attributable to WASH in Uganda. The application of the WADI device of HELIOZ with solar water disinfection (SODIS) improves the use of solar radiation by indicating the finalization of the disinfection process with a smiley.

The goals of this study comprise on one hand a contribution to the reduction of carbon emissions by replacing boiling as a water disinfection method by solar water disinfection (SODIS) and on the other hand the impact on different SDGs.

In the beginning a baseline-questionnaire is carried out to record the current situation in terms of water usage, water treatment, health, sanitation and hygiene. Water samples are taken and chemical contaminations, the microbiological quality and the impact of the SODIS/WADI treatment will be examined. 2000 WADIs are distributed to households and schools; and the users get trained on the use. As SODIS has shortcomings in turbid water BOKU experts develop application notes and pre-treatment of the water with appropriate means to manage the risk. The implication of the project will be determined at the end of the project by another questionnaire to estimate total CO<sub>2</sub> savings. The core part of the implementation is done in a participatory approach by the local partner Water School Uganda. BOKU experts select indicators for the SDGs, develop the questionnaire and educate local partners on sampling, chemical and microbiological monitoring, risk assessment, water pollution reduction and CO<sub>2</sub> pollution and reduction assessment. HELIOZ, who has experience from a previous SODIS/WADI project in Uganda, provides the WADIs and train the trainers on water, sanitation and health related issues together with Water School Uganda. Local government health workers will carry out regular household visits and increase awareness on water, sanitation and hygiene, supervision of follow up visits to the households will be ensured by Water School Uganda community workers.

#### **P 085      A Low-resource, Field-capable Assay to Detect Microbial Source Tracking Markers and Pathogens in Water**

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Identifying the source(s) of faecal pollution is critical to effective and timely water management and outbreak mitigation strategies. Traditional methods for microbiological pollution surveillance rely on screening faecal indicator organisms as surrogates for pathogens. However, results can take days, and in most cases, there is a lack of correlation between the presence of these microorganisms and those that are pathogenic to humans. Same day molecular methods have been developed that screen for host-specific markers which can detect and quantify faecal pollution. Yet, these methods are costly, require off-site processing and analysis using specialised equipment and necessitate a high level of technical expertise; this is not feasible for routine local monitoring. To address this, we developed a novel, low resource field-capable assay to detect microbial source tracking markers and molecular sequences of

waterborne pathogens. The dipstick technology, like an at-home pregnancy test, provides an indicator bar that correlates with the target microbe. While the application of this method to detect environmental microorganisms is novel, the concept of molecules competing for binding sites is already established in the fields of immunochemistry and proteomics. To date we have developed two proof of concept assays; 1) for the human specific *Bacteroides* spp. HF183 marker and 2) *Cryptosporidium parvum*. This approach steps away from the reliance on expensive equipment and technical expertise and can revolutionise water quality monitoring practices and catchment management strategies. Providing more definitive and timely data will not only improve environment protection services but contribute to better management and conservation of water ecosystems in real time.

**P 086 Tracking the sources of faecal contamination to stormwater constructed wetlands**

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Human health risks can result from contact with urban stormwater either during its use as an alternate water supply or during recreational activities. We still have very poor understanding of the sources of faecal contamination and their potential infectivity, which are important for QMRA and mitigation of the risk.

In this work, we studied a stormwater wetland which is used as part of a stormwater harvesting scheme to supply toilet flushing water and residential irrigation; this wetland has also been used for recreational activities. Our previous QMRA for this wetland, presented at WaterMicro2017, demonstrated that *Campylobacter* was driving the majority of risk in this system and that further treatment was required for any of these end-uses.

This current study selected 49 *Campylobacter* isolates from the inlet and outlet of the wetland; sequenced and conducted bioinformatics analysis to identify Multi-Locus Sequence Type (MLST). SourceAttribution modelling compared these isolates to local and worldwide database; and 25 of the 49 were identified as new MLST types. Of the 25, 5 had previously been isolated from infected humans, 19 were similar to types from waterfowl and 1 to a cattle source. To strengthen this evidence, source tracking using a 16S microbial community profiling method combined with the SourceTracker model which compares fingerprints of water samples from the wetland to those of animal and human faecal samples was conducted. This dataset reinforced that our samples were influenced by human sewage (0-4% of the community, average of 0.4%) and waterfowl faeces (0-42.2%, average 8%). This data not only provides stronger evidence to feed into new QMRAs to better understand the proportion of pathogens that are likely human-infective, they also provide some evidence as to the source of contamination in this system. The results of which will directly inform industry to target and mitigate specific sources of risk.

**P 087 Pepper mild mottle virus as an index of sewage pollution in shellfish and growing waters**

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Sewage contamination in shellfish and growing waters can have a negative impact on shellfish industry and public health. Current microbial quality assurance practice is based on faecal indicators, which are neither host specific nor predict the presence of enteric viruses. Similarly, detecting specific viruses from shellfish can be expensive and is not always straightforward. To address this issue, pepper mild mottle virus (PMMoV), a plant virus frequently present in sewage in high concentrations, was used to identify the sewage contamination in shellfish and growing waters. Faecal samples from non-human hosts ( $n = 104$ ), sewage samples ( $n = 10$ ), shellfish ( $n = 51$ ) and shellfish growing waters ( $n = 52$ ) were used to determine the host specificity and to identify the sewage contamination in shellfish and growing waters. Faeces of black swan, seagull, dog and Canada goose had PMMoV above the quantification level. PMMoV concentration in seagull faecal samples was higher ( $4.8 \pm 1.2 \log_{10} \text{GC/g}$ ) than dog ( $4.4 \pm 0.6 \log_{10} \text{GC/g}$ ) and black swan ( $3.3 \pm 0.1 \log_{10} \text{GC/g}$ ). All sewage samples were positive for PMMoV with the mean concentration of  $7.1 \pm 0.5 \log_{10} \text{GC/L}$ . The overall source specificity of the PMMoV was 0.92 (using LLOQ value). For shellfish, 46/51 (90%) samples were positive for PMMoV with concentrations between  $2.9\text{--}4.7 \log_{10} \text{GC/g}$ . For shellfish growing waters, 29/52 (56%) samples were positive for PMMoV with concentrations between  $3.7\text{--}5.8 \log_{10} \text{GC/L}$ . This is the first study that reports the presence of PMMoV in faeces of black swan and also the first study in New Zealand to report on the occurrence of PMMoV in the shellfish and growing waters. Despite the occurrence of PMMoV in certain animal/bird faecal samples, based on its high concentration in sewage, PMMoV may be a promising indicator of sewage contamination in shellfish and growing waters.

**P 088 Phages of *Bacteroides* spp. as a microbial source tracking (MST) tool for assessing drinking water sources in rural Kenya**

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This research is part of the OneHealthWater project and aims to develop microbial source tracking markers (phages of *Bacteroides* spp.) to investigate the importance of different human and cattle inputs of faecal contamination into rural Kenyan water sources.

Between May–November 2018, using a method adapted from Payan et al. (2005), potential human and cattle *Bacteroides* host strains were isolated from pooled faecal samples collected from school pit-latrines and from pooled cattle faeces, respectively. From 1349 bacterial colonies recovered on *Bacteroides* Bile Esculin (BBE) media, only 10 (5 human (KeHum 1 to 5) and 5 cattle (KeRum 1 to 5)) were presumptive *Bacteroides* spp. The novel *Bacteroides* hosts were then tested against municipal wastewater effluent from Nairobi. In addition, *E. coli* (EC), Intestinal enterococci (IE), somatic coliphages (SOMPH) and a previously isolated human-specific *Bacteroides fragilis* strain GB-124 (GB124PH) run in parallel to assess presence and levels of faecal contamination in 61 water sources (wells, boreholes, lakes, rivers, water pans and tanks).

Overall percentage and mean levels (CFU or PFU 100 ml-1) of faecal indicator organism in water sources were: EC (90.2%; 1,041), IE (91.8%; 1,421), SOMPH (47.5%; 241) and GB124PH (29.5%; 36). The percentage positive and mean phage levels using the most promising newly isolated human and cattle Kenyan strains from the water sources were respectively KeHum3 (21.3%; 34) and KeRum1 (26.2%; 61). Unfortunately, all potential Kenyan cattle markers also co-presented in municipal wastewaters from Nairobi (mean= 360 PFU 100 ml-1), although these levels were much below those of Kenyan and UK human markers (2,360) and GB124PH (7,800), respectively.

Results indicate that further research is needed in order to identify strains with a greater level of specificity, but the findings are encouraging in that low-cost phage-based MST methods has potential to identify faecal sources of contamination in the sub-Saharan region.

#### **P 089 How can we use faecal source-tracking as a tool to evaluate potential backup water resources?**

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Recent arid summers in Sweden has emphasised the need for a higher redundancy in drinking water production which may pose a challenge to municipalities. This is especially the case for incidents leading up to insufficient water quality or quantity in the primary water source.

Within an on-going case-study we aim to investigate the water quality in possible backup water resources and to evaluate the use of faecal source tracking as a tool for investigating the potential impact from effluents, agriculture and wildlife.

Mapping of potential contamination sources in main catchment areas of potential backup sources is performed through collecting data from public records with the help from the local municipality office. Microbial indicators (total coliforms, *E. coli*, *Clostridium perfringens* and *Enterococcus* spp.) and chemical parameters have been analysed in samples of surface water collected at the inflows in candidate water resources and at locations for potential raw-water intakes. Faecal source-tracking is performed using a library-based amplicon-sequencing technique targeting the V3-V4-region of the 16S rRNA gene. The reference library used consists of approximately 180 samples from 8 relevant faecal sources.

Preliminary results from samples collected during autumn 2018 indicate a relatively low level of contamination at the potential raw-water intakes while high levels of microbial indicators are sometimes seen in samples collected from inflows to the water catchments. Additional sampling of the water resources, to complete the case-study, are performed during spring and summer of 2019.

The case-study presented here is a part of an ongoing contingency planning project. The overall goal of the project being to spur discussion on the topic of back-up water resources and to encourage increased redundancy in drinking water production in municipalities in Sweden.

#### **P 090 Identifying the Source of Fecal Contamination in New Orleans East Canals**

Samendra Sherchan, Jia Xue

Tulane University

Storm water runoff is one of the most prominent non-point sources of contamination. The chemical and biological contaminants have the potential to degrade water quality and pose risks to human and ecosystem health. By monitoring fecal contamination using fecal indicator bacteria (FIB), we can prevent waterborne diseases. Although these FIB are present, it does not mean they are harmful; these bacteria indicate the possible presence of disease-causing pathogens. Therefore, identifying fecal

contamination in storm water runoff and outfalls is essential for remediation efforts to reduce the risks to public health. With the use of microbial source tracking along with the use of HF183 human fecal marker., we are able to characterize and confirm the human fecal contamination. This finding has a major health implication for those who may come into contact with contaminated water, in New Orleans East. 174 samples were collected over a year long period from four different canals throughout the Village de L'Est neighborhood in New Orleans East. The samples were analyzed through the use of DNA extractions and qPCR. Though the use of these methods, it was determined that 82.19% of the 176 samples tested positive for HF183. The canals of greatest concern, due to the high count of colony forming units, are those closest to the canal inlet: locations Dwyer and Einstein. This study was able to provide us with results that determined that the contamination being faced in the New Orleans East area is that of human, which will allow for proper remediation of those waterways effected.

**P 091 Human or Animal Waste? Determining the Sources of Fecal Pollution Using Innovative Molecular Methods in Surface and Groundwater**

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Presently, there is an incomplete understanding of the sources of pathogenic fecal contamination in karst aquifers such as the Edwards Aquifer in south-central Texas, which are vulnerable to human and animal fecal contamination. Understanding and identifying the sources of surface and groundwater fecal contamination is paramount to protecting water quality and implementing adequate pollution control and prevention strategies to mitigate pollution levels and risk to human health. Conventional fecal indicators such as *Enterococcus* and *Escherichia coli* are unreliable due to widely varying survival rates in the environment, failure to discriminate between sources, and inability to distinguish between fecal bacteria associated with recent contamination events and those adapted to secondary habitats. Microbial source tracking (MST) techniques can analyze water samples in a way that identifies and quantifies the source and species of fecal bacteria by targeting the Bacteroidales 16S rRNA genes using molecular techniques such as Polymerase Chain Reaction (PCR). Bacteroidales don't have ambiguous survival and sourcing issues and can provide good spatio-temporal resolution. The purpose of this study was to conduct fecal source tracking for the Recharge and Contributing Zones of the Edwards Aquifer in Bexar County, TX to resolve spatiotemporal patterns, seasonal variability, and identify sources of fecal contamination by applying PCR-based methods for seven primer sets (one universal, two human and four animal), two conventional fecal bacteria groups, *E. coli* and *Enterococcus*, and *E. coli* O157:H7, a standard for identification of a pathogenic sample. Water samples were collected at 21 locations consisting of wells, creeks and ponds beginning in January 2018 – December 2019. Results of this study will serve as a baseline for evaluating fecal microbial input, assess impact on water quality, human health and the environment, and assist in the development of BMPs for mitigating bacterial contributions to the water entering the aquifer.

**P 092 Gastropods as a source for the faecal indicator bacteria enterococci and *E. coli***

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The microbial safety of drinking water is routinely determined by testing for the presence of faecal indicator bacteria (FIB). Widely used indicators are *E. coli*, coliform bacteria and enterococci. Several practical cases in German water systems suggested gastropods as a source for the microbiological deterioration of drinking water. Therefore, we used a combination of cultural and molecular methods to test for the presence of faecal indicator bacteria in faeces of Gastropoda (slugs and snails of the

genera *Arion*, *Helix* and *Cepaea*).

All three groups of FIB were detected in Gastropoda by cultivation-based methods. Enterococci were present in most samples with a mean concentration of  $1.5 \times 10^6$  CFU/g faeces, with *E. casseliflavus* and *E. mundtii* as dominant species. Coliform bacteria, present in all samples, exhibited mean concentrations of  $6.3 \times 10^6$  CFU/g (main genera: *Citrobacter*, *Leclercia*, *Buttiauxella*, *Lelliottia*). *E. coli* was also detected, yet with lower concentrations of  $5.9 \times 10^4$  CFU/g. However, detailed specification showed that the majority of these putative *E. coli*-isolates could be assigned to the genus *Buttiauxella*. We could show that this *Buttiauxella* strain exhibits  $\beta$ -glucuronidase activity, explaining the false-positive results with the standard ISO-methods. Accompanying phylogenetic analyses of the complete microbiome of the samples confirmed the cultivation results and the presence of FIB in faeces of Gastropoda. *Enterobacteriaceae* were dominant in the samples, yet only a small number of sequences could actually be assigned to the genus *Escherichia*. Thus we conclude that enterococci and coliform bacteria are an integral component in the microbiome, whereas *E. coli* might be derived from other faecal sources with gastropods being a vector.

The results show, that the current concept of faecal indicator bacteria needs to be extended, as not only humans and homeothermic animals could be a source for FIB, but also Gastropods and other invertebrates need to be taken into consideration.

#### **P 093      Can become microbial indicators and stable isotops a suitable combination to identify the source of nitrate in waters?**

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The presence of nitrates is the most important diffuse pollution problem and responsible for the poor ecological state of groundwater bodies. The origin of nitrate in water is mainly attributed to agricultural and livestock activity (application of inorganic fertilizers or manure/slurries), wastewater discharges and septic tanks. Microbial indicators have been evaluated to detect the origin of faecal contamination in water. On the other hand, numerous studies corroborate the usefulness of stable isotopes of elements such as N, C and S as tracers of pollution sources.

In this work we have combined the analysis of microbial indicators with stable isotopes to classify the origin of nitrate into inorganic and / or organic and identify the source in the latter case. A total of 18 samples of treated sewage, 39 surface water samples and 148 groundwater samples from different wells were analyzed for a) physical-chemical parameters, b) the isotopic composition of the N and O of nitrate ( $d_{15}\text{NNO}_3$  y  $d_{18}\text{ONO}_3$ ), the isotopic composition of boron ( $d_{11}\text{B}$ ), and the isotopic composition of S and O of sulphate ( $d_{34}\text{SSO}_4$  i  $d_{18}\text{OSO}_4$ ), and c) general microbial indicators of faecal pollution: *E. coli*, Enterococci and somatic phages; and some host associated source tracking markers: human (GA17PH, HMBif and crAssphage), pig (Pig2Bac and Pomito), ruminant (CWBif) and poultry (PLBif). At the same time, we performed a thorough hydrogeological characterization and analysis of the land uses of the surrounding sampling areas.

Combining the isotopic and the microbiological indicators data, it has been possible to define the origin of nitrate at each point studied. A complete or partial correlation between the two kinds of parameters (chemical and microbiological) has been observed for the 79% of the samples confirming that the two types of analysis are complementary providing reliable information on the origin of the contamination.

**P 094      Novel Multiplex Microfluidic Device for Microbial Source Tracking Targets Takes the Lab to the Field**

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Outbreaks of waterborne diseases remain a global public health concern, not only by the morbidity and mortality that they cause, but by the high cost of prevention and treatment. Microbial source tracking (MST) techniques are used more frequently as powerful technique to identify the origins of faecal pollution in water. Distinguishing between pollution from animal and human faecal origin is important for risk management.

Common MST markers include human and animal gene targets in *Bacteroides* and recently the *Escherichia coli* H8 gene. These bacterial genetic targets have been employed in MST studies using amplification techniques such as the polymerase chain reaction (PCR) and quantitative PCR approaches. While these techniques have led to significant progress in MST studies, key obstacles such as the need of labeling, high costs, slow turnaround-time, and the complicity of operation have limited their applications. Thus, there's an urgent need to design de-novo devices for improved and rapid detection of microbial nucleic acids for many purposes, including water quality monitoring and public health protection.

Microfluidics technology, i.e. handling of microscale fluid samples, has found various applications, particularly within nucleic acid analysis in microbiological assays. The advantages of microfluidics, such as portability, automation, and high-throughput, have opened new avenues for identifying the source of microbial contaminants in water.

Herein, we report development of a simple, low-cost microfluidic device with an array of reactors, which are pre-loaded with different primer pairs for simultaneous real-time PCR-based detection of MST markers, *Gen Bac III, Uid A, and E. coli H8*. The chip operation comprises a single-step manual pipetting step, which can be deployed in resource limited setting. The temperature of the microPCR-chip is controlled by a custom-built portable and programmable PCR apparatus. We believe that this device offers simple and cost-effective platform to accelerate the detection and identification of waterborne pathogens.

**P 095      Reduction of crAssphage and enteric viruses during conventional wastewater treatment**

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Wastewater treatment plants (WWTP) are currently facing numerous issues regarding inadequate removal of the pathogenic viruses. The conventional bacterial fecal indicator during treatment is insufficient to indicate the presence of all pathogenic viruses, because the reduction efficiency of these bacteria is relatively higher than pathogenic viruses. In this context, using the reclaimed water with occurrence of pathogenic viruses might outgrowth the threat to the human health. Worldwide, many studies have been conducted regarding the reclaimed WWTPs efficacy but it showed that highly sophisticated treatment system currently deployed is still struggling to remove the pathogenic viruses. There are other quantitative Polymerase Chain Reaction (qPCR) assays that have been developed based on viruses, for detecting human fecal pollution. But their concentrations are still detected lower than the HF183 which is a human specific and widely used in microbial source tracking studies. In contrast, some of these viruses (pepper mild mottle virus, coliphage) have additional advantages including non-pathogenic characteristics and can be measured quickly using qPCR assays. A new crAssphage qPCR assay has been recently developed to quantify human fecal pollution in environmental waters. This DNA bacteriophage "crAssphage" was recently discovered and is more

prevalent and abundant in human feces and sewage. However, there are only limited studies understanding the role of this bacteriophage in wastewater reclamation. The objective of this study was to evaluate this novel crAssphage marker for assessing the removal of viruses in wastewater treatment from New Orleans, LA. CrAssphage was detected in all samples with a high frequency 13 (100%) out of 13. The mean concentration of crAssphage in untreated sewage was remarkably high,  $10.3 \pm 1.3 \log_{10}$  copies/L.

**P 096 Flies and stagnated water as two major human-associated fecal transmission pathways in peri-urban communities of Lusaka, Zambia**

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In Zambia, frequent cholera outbreaks have recently been reported mostly in heavily-populated peri-urban areas; however, the fecal transmission pathways have not yet been well understood. This study aimed to identify the potential major fecal exposure pathways and track their contamination sources. Field tests were conducted in both dry and rainy seasons (Aug-Sep 2018; Feb-Mar 2019). Two peri-urban slums of Lusaka city, Zambia were selected for this study. Firstly, microbial contamination data was obtained from various environmental samples ( $n=1-20$  in each sample group; 16 groups) as potential transmission media via *Escherichia coli* tests. For the sampling method, particularly for flies, they were collected by using sticky fly tapes. Secondly, host-associated genetic markers were applied for *E. coli* isolates (H8 for human), and for DNA extracted from filtered samples (HF183 for human; GFD for avian). The results of this study highlight high *E. coli* contamination levels in flies (0-2890 cfu/fly; 1-10 flies per trap) and stagnated water (0-26100 cfu/mL) in rainy season. Among these samples, they were mostly contaminated by human feces. In terms of H8, flies outside toilet entrance, inside toilet space, near household entrance, inside kitchen and stagnated water had 100%, 100%, 73%, 33% and 83% H8-positive results respectively; for that of HF183, they were 100%, 18%, 14%, 100% and 50% HF183-positive respectively. There were no GFD-positive results observed. These findings suggest that flies and stagnated water may be the major human-associated fecal transmission pathways which can be useful in planning policies and designing suitable interventions in reducing public health risks.

**P 097 A Possible Breeding Ground for Emerging Pathogens?**

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The increased occurrence of antimicrobial resistance (AR) in microorganisms is receiving more attention with the WHO putting efforts into monitoring the AR of selected bacteria worldwide. To determine the presence of diarrhoeal pathogens, specifically pathogenic *Escherichia coli* (*E. coli*) and their antimicrobial resistance profiles, the spotlight is on wastewaters which are known to be a hot spot for a variety of microbial populations. *E. coli* contains pathogenic and non-pathogenic strains and adequate identification is needed to determine the implications and effects of the presence of the bacteria. Even though a total gene combination need not be present for classification of the different pathogens, a growing trend in the presence of different gene combinations have been noted. The presence of these different genes may create super bacteria which may have the ability to be antibiotic resistant. Studies have shown that antibiotic resistance found in bacteria, originated from environmental settings. Determining the antibiotic resistance of these strains will assist in risk analysis and management especially in rural communities who are dependent upon these waters for their daily needs.

Water samples were collected upstream and downstream of the treated effluent release point and from the raw sewage after primary settling. Samples were enriched on selective media and all *E. coli* isolates present screened for the presence of virulence genes, antimicrobial susceptibility.

Faecal matter may contain intact antibiotics and/or residues which end up in wastewater. The positive *E. coli* isolates show the presence of multiple virulence genes within a colony suggesting the emergence of possible new strains of *E. coli* bacteria. The development of antibiotic resistance is believed to be developed within the wastewater treatment plant since a trend in the antibiotic resistance pattern between the wastewater samples and the environmental samples can be drawn.

**P 098      A study into tidal variation on bathing water quality located on rural beaches in County Dublin**

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Bathing water quality is monitored all year around to ensure the health and safety of the public in accordance with the EU Bathing Water Directive 2006/7/EC. The presence of faecal pollution in bathing waters is of huge importance regarding public health risks. It is crucial to identify the origin of contamination to design tools to minimise their impact. However, the identification of faecal pollution sources is not easy because diffuse contamination from livestock and seabirds prevails in these rural areas.

This study was carried out on 'poor' and 'excellent' categorized bathing waters, The Brook Beach, Portrane and Balcarrick Beach, Donabate beach, respectively, during the bathing season to identify drivers that impact faecal indicator organism (FIO) levels in bathing waters.

Two transect studies were performed on each beach over a 12 hour tidal cycle, commencing at high tide sampling every 30 minutes; following the tide out and back in again. Samples were analysed for levels of *E. coli*, intestinal enterococci, somatic coliphages and *Clostridium perfringens* spores. Microbial source tracking (MST) was used to identify human, seagull, ruminant, dog and horse faecal contamination. Based on these results, a second study was carried out in Rogerstown estuary to determine the levels of FIO entering and leaving the estuary in a tide dependent manner to determine whether this may impact on these beaches.

Our current data showed that FIO levels vary by one order of magnitude on both Portrane and Donabate beaches at the compliance points however further up on Portrane beach a pattern with tide and rising levels of FIO can be seen. The estuary study indicates a clear tidal impact from the estuary on FIO levels on Portrane bathing water. All MST markers were detected on both beaches indicating that bathing water is a complex environment which requires further study.

**P 099      Relative decay of sewage-associated marker genes and traditional fecal indicator bacteria in recreational water and sediment**

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Exposure to recreational waters contaminated by human sewage poses potential human health risks. Microbial source tracking (MST) markers are increasingly being used to detect cases of sewage contamination and inform recreational water management decisions. While levels of health risk are

known to diminish as sewage ages in the environment, little is known regarding the environmental decay rates of many commonly used MST markers and whether they accurately reflect likely levels of health risk. This knowledge gap creates uncertainty for water quality managers. In this study, we investigated the decay of fecal indicator bacteria (*E. coli*, enterococci), *Bacteroides* HF183, human adenovirus (HAdV), human polyomavirus (HPyV), and crAssphage in outdoor mesocosms containing fresh and marine waters and their corresponding sediments. Decay rates of FIB and HF183 were fast and similar in water samples across the three mesocosms. Similarly, decay rates of viral targets (HAdV, HPyV and crAssphage) were similar in water samples, however, viral targets persisted significantly ( $p < 0.05$ ) longer in water samples than did bacterial targets. Decay rates of FIB and sewage-associated markers in sediment mesocosm samples varied considerably across the marine and freshwater mesocosms. Decay rates of FIB and sewage-associated markers were significantly faster in water samples compared to sediment in all mesocosms. FIB exhibited biphasic decay rates compared to MST markers in water and sediment samples from all mesocosms. Decay rates of FIB and MST markers significantly and positively correlated with mesocosm water temperature, total organic carbon, and turbidity, and were mostly negatively associated with rainfall, electrical conductivity, evaporation, sunlight, and pH. The results indicated that care should be taken when interpreting the occurrence of FIB and sewage-associated MST markers in water, which may originate from resuspended sediments. The differential decay of these targets are likely to impact on risk interpretation and management decisions.

**P 100 A duplex PCR assay quantifies *Bacteroides* HF183 and crAssphage CPQ\_056 marker genes in environmental waters**

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The use of microbial source tracking (MST) marker genes has grown in recent years due to the need to attribute point and non-point fecal contamination to specific sources. The *Bacteroides* HF183 and crAssphage marker genes are highly abundant in untreated wastewater and have been widely used to identify sewage contamination in catchments waters. These two marker genes are highly associated with sewage We developed a duplex quantitative PCR (qPCR) assay for simultaneous quantification of HF183 and crAssphage CPQ\_056 marker genes in raw wastewater and stormwater samples. Published simplex qPCR assays were upgraded to form a duplex qPCR assay. The performance characteristics of the duplex qPCR assay were rigorously evaluated and were similar to its simplex counterparts. The performance of the duplex assay was validated in the laboratory where it was developed and as well as in a collaborative laboratory study with the aim to evaluate reproducibility, sensitivity and accuracy of the assay to quantify these marker genes in storm water samples. The concordance values between the simplex vs. duplex qPCR assays for HF183 and crAssphage CPQ\_056 marker genes ranged from 96.7-100%, and the detection rate and mean concentrations of HF183 and CPQ\_056 in environmental water samples were remarkably similar for the duplex qPCR assay suggesting the reliability of this assay for monitoring HF183 and CPQ\_056, simultaneously. This assay based on quantification of two marker genes will minimize the risk of false negative results in environmental water samples contaminated with low levels of sewage. In addition, this assay will facilitate rapid analysis of water samples for the presence of sewage contamination with reduced cost and increased certainty.

**P 101      Loss of DNA to surface-reactive matrix compounds during extraction - the need for comprehensive process controls in molecular diagnostics of water samples**

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Since routinely applied standard fecal indicator bacteria do not provide information on the pollution source, alternative indicators, most prominently host-associated genetic bacterial 16S rDNA markers, have been developed (Microbial Source Tracking). Despite the advantage of being very sensitive and specific, application of these methods is often limited by the efficiency of DNA extraction. The present study evaluated the use of an operational control to investigate effects of matrix interferences during extraction. The aquatic habitat studied was Lake Neusiedl in the Eastern part of Austria, an important recreation area for the urban area of Vienna. The water matrix was characterized by high and fluctuating levels of salts, humic acids, and suspended solids (TSS). Samples were spiked with a defined target cell standard (DeTaCS) and a strict evaluation of PCR-inhibition using an internal amplification control and different dilutions for each sample was performed. DNA was extracted using bead-beating in combination with a phenol-chloroform and CTAB based method. Results demonstrated that inhibition of the PCR reaction played a minor role but that the presence of high amounts of TSS led to a complete loss of DNA (i.e. recovery rates  $\leq 1\%$ ). To test the hypothesis that the adsorption of DNA to surface-reactive matrix components was the causing mechanism, experiments with different amounts of sodium pyrophosphate and salmon sperm DNA as co-precipitants were conducted. Addition of these adsorption site competitors helped to recover DNA concentrations to near control levels. The results from this study clearly showed the need for a stringent process control on a sample-to-sample basis since extraction efficiency varied not only spatially but also temporally. The appropriate choice of an extraction method suitable for a particular sample matrix and the application of a sample process control as prerequisite for successful downstream analysis, is compulsory for accurate source identification and human health risk assessment.

**P 102      Pepper mild mottle virus and crAssphage as fecal pollution markers in aquatic environments of the Kathmandu Valley, Nepal**

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Numerous human-specific viral markers have been designed to track the presence of human fecal contamination in aquatic environments; however, they lack adequate sensitivity and specificity in different geographical regions. This study aimed to evaluate the performances of six human-specific viral markers (Aichi virus 1, human adenoviruses, BK and JC polyomaviruses, pepper mild mottle virus, and crAssphage) using 122 fecal-source samples collected from humans and five animal hosts in the Kathmandu Valley, Nepal. Pepper mild mottle virus and crAssphage showed high sensitivity (90–100%) with concentrations of 4.5–9.1 and 6.2–7.0 log copies/g wet feces ( $n = 10$ ), respectively, whereas human adenoviruses, BK and JC polyomaviruses, and Aichi virus 1 showed poor performances with sensitivities of 30–40%. Pepper mild mottle virus and crAssphage were detected in 40–100% and 8–90%, respectively, of all types of animal fecal sources and showed no significantly different concentrations among most of the fecal sources ( $P > 0.05$ ), suggesting their applicability as general fecal pollution markers. Furthermore, 115 environmental water samples were tested for pepper

mild mottle virus and crAssphage to identify fecal pollution. Pepper mild mottle virus and crAssphage were successfully detected in 62% (71/115) and 73% (84/115) of water samples, respectively. The greater abundance and higher mean concentration of crAssphage ( $4.1 \pm 0.9$  log copies/L) compared with pepper mild mottle virus ( $3.3 \pm 1.4$  log copies/L) indicated greater chance of detection of crAssphage in water samples, suggesting that crAssphage could be preferred to pepper mild mottle virus as a marker of fecal pollution.

**P 103      Detection of Rotavirus and Risk Assessment of Faecal Contamination Using Bacterial and Viral Indicators in a Surface Water in Nigeria**

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This study was aimed at determining whether bacterial and viral faecal pollution markers may provide a better indication of human health risks associated with rotavirus in a surface water in Akure, Nigeria. Water samples were collected from River Ala in Akure metropolis on a weekly basis over a period of 12 weeks (n = 12). Levels of faecal indicator bacteria were determined using membrane filtration technique and those of somatic coliphages were determined by standardized direct plaque assay. The detection of rotavirus was determined using molecular technique. Physicochemical characteristics of the water samples were determined using standard methods. The results revealed that the concentration of *E. coli* in the water samples ranged from  $3.19$  to  $4.09$  log<sub>10</sub> CFU/100 ml, faecal coliforms  $3.53$  to  $4.35$  log<sub>10</sub> CFU/100 ml; intestinal enterococci  $2.92$  to  $3.68$  log<sub>10</sub> CFU/100 ml; *Salmonella*  $2.80$  to  $3.26$  log<sub>10</sub> CFU/100 ml; somatic coliphages  $2.52$  to  $3.31$  log<sub>10</sub> PFU/100 ml; and rotavirus ranged from zero to  $2.17$  log<sub>10</sub> genome copies per 100 ml. Whilst rotavirus was detected on only two sampling occasions, somatic coliphages were detected on all sampling occasions. *Salmonella* demonstrated significant correlation with rotavirus ( $r = 0.42$ ). *E. coli*, faecal coliforms and somatic coliphages were observed to exhibit inverse relationship with electrical conductivity, whereas rotavirus showed a negative relationship with turbidity and positive relationship with water hardness. The findings from this study demonstrated that *Salmonella* and somatic coliphages may be used as indicators of human health risks associated with rotavirus in faecally impacted surface waters. Physicochemical factors such as electrical conductivity, water hardness and turbidity may also be used for modelling and evaluation of hazards in water quality monitoring for human health protection.

**Keywords:** rotavirus, human health, faecal contamination, somatic coliphages, surface water

**P 104      Successful Application of Microbial Source Tracking Using GB-124 Bacteriophage as an Indicator of Human Faecal Contamination in Environmental Samples in Kolkata, India**

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Microbial source tracking (MST) using bacteriophages infecting certain *Bacteroides* spp. has been proposed as a reliable method for indicating human faecal contamination and to examine the risk of exposure to pathogens of human origin. The objective of this study was to investigate the abundance of phages capable of infecting existing strains- *B. fragilis* (GB-124), *E. coli* (WG-5) and a newly isolated strain *K. intermedia* (ASH-08) in a range of human and non-human samples from Kolkata, India. We tested 56 samples of human origin (28 during the 2018 rainy and 28 during the 2017/18 dry season). Human samples included municipal sewer waters collected from manholes, and canal/drain waters known to be impacted by human waste. In addition, 60 faecal samples from a range of animal species were tested to determine human specificity in this setting.

Our results showed that *Bacteroides* GB-124 phages were present in 26/28 (93%) and 20/28 (71%) of samples collected during rainy and dry season, respectively. The mean phage concentration ( $\log_{10}$  PFU/mL) ranged from 1.37 (max = 2.35) during the rainy season, to 1.69 (max = 3.60) during the dry season, and the difference was significant ( $P$ -value < 0.05). Phage capable of infecting strain GB-124 were absent from 95% of animal faecal samples, though they were present in three pig samples. Somatic coliphage and phage infecting ASH-08 were present at concentrations of up to 5.58 and 5.15  $\log_{10}$  PFU/mL in wastewater, respectively and up to 7.21 and 7.00  $\log_{10}$  PFU/g in animal faeces, respectively. Our results suggest that strain GB-124 may offer an effective phage-based technique to differentiate contamination sources in Kolkata, India. As such, GB-124 phage can provide specific, quantitative, low-cost, culture-based MST in an urban setting with dense human and animal populations.

**P 105      MALDI-TOF MS identification of microbial contaminations in drinking water systems**

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Widely used indicators for the determination of the hygienic quality of drinking water are *E. coli*, coliform bacteria and enterococci. While *E. coli* specifically indicates fecal contaminations, coliform bacteria or enterococci have also been isolated from environmental sources like plants, invertebrates or sediments. If indicator organisms are detected in drinking water systems, the reasons or sources for these contaminations are often unknown.

Fast and specific methods for the identification of indicator bacteria are helpful to identify the sources of the contaminations. In addition, the correct and fast identification of a microorganism is crucial for further decisions and measures like e.g. boiling advices or disinfection measures.

MALDI-TOF MS is an identification method based on bacterial protein spectra and is routinely used for the identification of clinically relevant bacteria. Due to fastness and reliability, the method is increasingly used also for isolates from water environments.

In our study, we use MALDI-TOF MS for the identification of indicator bacteria (mainly *Enterobacteriaceae* and enterococci) from various water environments, like drinking water or surface water and compared the results with PCR-based methods. These include RAPD-PCR as a fast typing method and amplification and sequencing of the 16S rRNA gene and specific marker genes. Furthermore, the resolution of the MALDI-TOF MS method was investigated by clustering analysis of the spectra of closely related strains.

Our results indicate that MALDI-TOF MS is in principle a suitable method for the identification of indicator bacteria from water. Due to the fastness, MALDI-TOF MS has the potential to become the routine identification method also in the drinking water sector. Yet, the current database is not sufficient to correctly identify all relevant strains within the *Enterobacteriaceae* and enterococci, as the database is still dominated by clinically relevant bacteria and many environmental isolates are missing.

**P 106 Recovery efficiencies of the crAssphage genetic marker for human-specific source tracking in wastewater and environmental water**

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The process of pinpointing water pollution sources using host-specific gastrointestinal microbes, known as microbial source tracking (MST), has significant benefits for countries with water quality management issues related to pollution. A bacteriophage recently discovered using a metagenomic cross-assembly approach, or crAssphage, shows promise as a means of tracking human-specific fecal pollution. Molecular methods, including quantitative PCR and digital PCR, have been developed for quantitative detection of the crAssphage genetic marker; however, loss of genetic materials during the recovery and detection processes could alter the quantity of virus in a water sample. This study aims to determine the crAssphage marker's recovery efficiencies in different water sources, including influents (WWTPinf) and effluents (WWTPeff) from municipal wastewater treatment plants, as well as lightly and heavily polluted beach water and freshwater. Because crAssphage cannot be propagated in laboratory settings yet, concentrated stock from polluted canal water was used as a spike. Spiked water was processed through pre-acidification to pH 3.5 prior to vacuum filtration with 0.45 µm-pore-size HAWP membranes. The filtered membranes were DNA-extracted using a commercial kit and DNA extracts were stored at -80°C until use. The CPQ\_056 qPCR primers and hydrolysis probe were used to quantify crAssphage gene copy numbers. Recovery percentage was calculated as a ratio of crAssphage gene copies in a final spiked DNA extract and those in stock for spiking. Indigenous crAssphage gene copies in water were also quantified and subtracted before calculating recovery efficiencies. The results in triplicates demonstrated low recovery in less-polluted water, to high recovery in polluted water. The results help to correct data bias related to studies across geographical areas and increase data accuracy for further quantitative microbial risk assessment (QMRA).

**P 107 Towards an Ultrasound Enhanced Assay using Attenuated Total Reflection Infrared Spectroscopy for Detection of Bacteria in Drinking Water**

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In this work, we present our efforts towards the combination of an acoustic trap and attenuated total reflection (ATR) Fourier-transform infrared (FTIR) spectroscopy to perform an ultrasound (US) enhanced assay for the rapid detection of bacteria in drinking water. In recent years, the manipulation of mammalian cells, yeast and bacteria via ultrasound particle manipulation for applications like cell sorting and enhanced spectroscopy has been demonstrated. By mounting an acoustofluidic cell on top of a custom-built ATR setup, we were able to trap bacteria by relying on so-called ultrasonic radiation forces, without the need of mechanical retention elements. A dedicated liquid handling system was employed for automated and reproducible sample handling and bacteria retention in the acoustic trap. To showcase the potential of the presented setup for monitoring microbial pollution in water, we monitored *Escherichia coli* suspensions at different concentrations. Preliminary experiments were performed by retaining bacteria in the acoustofluidic cell followed by pumping an enzyme-labeled antibody solution into the cell. Subsequently the bacteria-antibody conglomerate was supplied with enzyme substrate and the conversion was monitored via ATR-FTIR spectroscopy. Throughout the entire liquid-handling sequence, bacteria were stably retained in the cell allowing for elegant liquid exchange of the solutions involved in the assay procedure. In contrast to common direct enzyme-linked immunosorbent assays (ELISA), no immobilization of *E. coli* on a surface like 96-well plats is needed. Furthermore, the inherent acoustic streaming occurring in parallel with ultrasonic radiation forces reduces the antibody-antigen incubation time significantly resulting in an overall assay time of approximately 60 min. This proves the potential of ultrasound enhanced assays for the detection of microbial pollution in water.

## **POSTER SESSION WEDNESDAY**

### **P 108 Applying flow cytometry as a monitoring tool for drinking water management**

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Microbial monitoring of drinking water is of great importance to guarantee high quality water and to mitigate health hazards. Flow cytometry (FCM) is a fast and robust method to determine bacterial concentration in liquids. In this study, FCM was applied to monitor the microbial communities and dynamics in a drinking water distribution system (DWDS), after implementing ultrafiltration (UF) combined with coagulation at the WTP Kvarnagården in Varberg, Sweden. The new treatment process led to both a drastic reduction of total cell counts (TCC) by a factor of  $10^3$  cells/mL; and, increased removal rate of organic matter by 50 % in outgoing drinking water. In this context, FCM monitoring was initiated to study the impact of the changed water quality on the biofilm within the DWDS. Over a period of twelve months, water samples were analyzed to identify typical baselines for specific areas in the network with hydraulic and seasonal variations.

The data analysis showed that different factors have impact on the biofilm conditions. Besides specific distances from the WTP, it is most likely that the retention time in the DWDS has the greatest impact on the TCC, which was generally higher at sampling spots with long retention times. During spring, TCC ranged from  $3 \times 10^2$  cells/mL to  $1.1 \times 10^5$  cells/mL for various sampling spots and variations of the microbial community were observed. The increase of water temperature during the summer period resulted in up to 5.5-fold increases of TCC and changes in the microbiome at almost all sampling spots. The annual TCC profiles showed a clear seasonal course and pattern over time. The obtained FCM baselines will provide an important foundation for monitoring and evaluation of the next significant change at the WTP, the gradual removal of chloramine.

### **P 109 Assessment of electrocoagulation as a tertiary treatment in removing enteric pathogens and antibiotic resistant bacteria from municipal wastewater**

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Electrocoagulation is an electrochemical technology with a great potential to treat domestic effluent with a wide range of pollutants such as microorganisms, organic micro pollutants, nutrients and heavy metals. In this research, a low voltage (<30V) laboratory scale electrocoagulation batch unit equipped with high purity iron plates was used to evaluate the removal of pathogenic organism indicators, Antibiotic Resistant Bacteria (ARB), Antibiotic Resistance Genes (ARG) and nutrients from synthetic secondary wastewater treatment plant effluent for prospective reuse applications. Several combinations of charge dosage (from 50 to 350 C/L) and charge dosage rates (5, 7.2, 36 and 72 C/L/min) were used, as well as varying concentrations of nutrients in the synthetic effluent. Challenge micro-organism *E.coli* strain WR1 and somatic coliphage Phi-X174 removal reached 4.96 log and 5.40 log, respectively, under charge dosages of 200 C/L and 345 C/L (58.04 and 100 mgFe/L) dosed at 36C/L/min and 7.7C/L/min respectively. Phosphorus removal increased with increasing charge dosage, with a minimum removal of 68% at 50 C/L to nearly 100% for dosages above 150 C/L. Conversely, nitrogen removal decreased with increasing charge dosage; from a maximum removal of 92% at 50C/L, to negligible in the proximity of 200C/L. Iron remaining in the supernatant was very low, with a minimum removal (relative to the dosage) of 87% for 50C/L and over 99% for 150 C/L and above (all values in the 0.1 – 1.3 mg Fe/L). Removal efficiency testing for ARB, ARG and *Clostridium* spores is currently underway.

**P 110      Understanding the enhanced removal of PPCPs by aerobic granular sludge under different C/N ratio**

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In recent years, pharmaceuticals and personal care products(PPCPs) as one kind of emerging pollutants, have been detected frequently in Wastewater treatment plants(WWTPs) effluent and natural waters, which affect the safety of water ecology and health of human seriously. In most of WWTPs, the C/N ratio is an important parameter for pollutants removal, and it affects the enrichment of microbes with different growth rates and nutrition types. Thus, three aerobic granular sludge reactors with different C/N ratios were built in this study, and the effect of two typical PPCPs(roxithromycin, ROX; sulfamethoxazole, SMZ) on the properties of aerobic granule and their removal performance were investigated. Results showed R3 reactor under condition of low COD/TN continued to be stable for more than 2 months after PPCPs addition, and the average granular sludge size reached  $1268\pm21 \mu\text{m}$ . The abundance of tyrosine protein, tryptophan protein and hydrophobic humin acids in sludge EPS were relatively high, which maintains the stability of granular structure. The removal efficiency of ROX and SMZ in R3 was  $95.2\pm0.5\%$  and  $92.9\pm0.3\%$  respectively, which were obviously higher than those in R1 and R2 with high C/N ratio. Further analysis of sludge microbial community found that at the genus level, the dominant bacteria in R3 granular sludge were Thauera spp. (31.4%), Rhodocyclaceae spp. (6.4%), Xanthomonadaceae spp. (5.8%), Sphingobacteriales spp. (4.3%) and Nitrospira spp. (3.2%), given the system better secretion of EPS, denitrification and PPCPs degradation. Comparatively, the proportions of Defluviicoccus spp. and Chryseolinea spp. increased in the presence of ROX and SMZ, which affects the stability of granular sludge.

**P 111      Ultraviolet (UV) Light Emitting Diode (LED) Inactivation of *Ascaris lumbricoides* Ova**

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Asian Institute of Technology (AIT); Swiss Federal Institute of Aquatic Science and Technology (Eawag)

*Ascaris lumbricoides* is an intestinal parasite responsible for roundworm infections in 1.4 billion people worldwide. With a fecal-oral transmission pathway, *ascaris* are pathogens of concern for wastewater reuse for agriculture in developing economies. The World Health Organization (WHO) requires less than one intestinal nematode egg per liter for treated wastewater used for crop irrigation.

This work investigated *Ascaris lumbricoides* inactivation by low pressure (LP) ultraviolet lamps emitting at 254 nm and UV light emitting diodes (LEDs) emitting at 280 nm. Wild type *Ascaris lumbricoides* eggs harvested from the feces of human patients by the Office of Disease Prevention and Control in Chiang Mai, Thailand, were isolated through centrifugation, drained, and resuspended in a salt solution to preserve their viability.

Samples of 500 eggs/mL suspended in phosphate buffered saline were exposed to 254 nm and 280 nm light at doses up to 400 mJ/cm<sup>2</sup> in 50 mJ/cm<sup>2</sup> increments. *Ascaris* viability was measured following the Standard Methods for Recovery and Enumeration of Helminth Ova in Wastewater, Sludge, Compost, and Urine-Diversion Waste in South Africa. Samples were centrifuged, resuspended in sulfuric acid to mimic the conditions of a human stomach, and incubated at room temperature for 28 days. The eggs were characterized by their development as undeveloped fertilized eggs, partially developed ova, or developed worm larvae.

LP UV emitting at 254 nm was more effective than the LEDs emitting at 280 nm. LP UV resulted in an inactivation rate constant matching the literature of approximately 0.0078 cm<sup>2</sup>/mJ compared to the inactivation rate of 0.004 cm<sup>2</sup>/mJ for the 280 nm LEDs. This is expected given that UV-induced DNA damage is the dominant inactivation mechanism at these wavelengths and DNA absorbs 254 nm more strongly than 280 nm. This work showed that while *ascaris* are highly resistant to UV, they are still susceptible to inactivation.

## P 112 Solar-powered point-of-use drinking water treatment in rural Kenya

Samuel Dorevitch, Abhilasha Shrestha

University of Illinois at Chicago School of Public Health

Decentralized point-of-use (POU) water disinfection may help meet the Sustainable Development Goal (SDG) target 6.1: "By 2030, achieve universal and equitable access to safe and affordable drinking water for all." Several POU disinfection methods have been evaluated as means of reducing the incidence of childhood diarrhea, though all have limitations and relative advantages. We describe the evaluation and application of a new POU method of drinking water treatment that uses a solar powered ozone (O<sub>3</sub>) generator. The PO<sub>3</sub> system was assessed in a series of studies. Initial work focused on the assessment of the ability of the PO<sub>3</sub> system to reduce bacteria and virus concentrations in wastewater. This showed microbe reductions of approximately 2-log orders, though the reductions varied by treatment time, turbidity, and volume of water treated. The method was next tested in Western Kenya at the laboratory facility of an NGO that focuses on WASH. Operated on solar power, the PO<sub>3</sub> units were able to reduce *E. coli* concentrations of approximately 2,000 MPN/100mL in very turbid water (>100 NTU) down to approximately 10 MPN/100mL. Next, solar panels and PO<sub>3</sub> units were provided to 10 families in a Kenyan village that lacks running water and electricity. Significant improvements in the quality of household stored drinking water were observed. Results of focus group discussions with users of the PO<sub>3</sub> units indicate a high level of enthusiasm for the system. This novel approach to POU water treatment needs to be evaluated further, as it showed promise in the studies described.

## P 113 Use of copper alloys to prevent and control biofilm formation in plumbing systems: action of chlorine and flushing

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LEPABE, Department of Chemical Engineering, Faculty of Engineering, University of Porto/CEB-Centre of Biological Engineering, University of Minho, Campus de Gualtar

Copper pipes are conventionally used to supply tap water. However, their role in biofilm prevention remains to be understood. This study evaluates the ability of selected surface materials with different copper contents (0, 57, 79, 83, 96, 100% of copper) to control biofilm formation and regrowth, without disregarding copper leaching and corrosion under conditions mimicking real plumbing systems. *Acinetobacter calcoaceticus* and *Stenotrophomonas maltophilia* isolated from drinking water were used as model bacteria for biofilm formation for 24 and 48 h. All the copper materials showed positive results on biofilm prevention (reduction > 4 log CFU/cm<sup>2</sup>). The alloy containing 96% of copper was the most promising surface and was the only surface able to avoid regrowth of single species biofilms. The alloy with 83% copper demonstrated to be unsuitable for the use in chlorinated systems due to the high copper leaching in the presence of chlorine. The alloys with the best performance in 48 h-old biofilm control (57 and 96% copper) were further tested in a rotating cylinder reactor (RCR). Biofilms were grown for 7 days and exposed to three different treatments: chlorine (10 mg/l; 10 minutes), increased shear stress (fluid velocity - 1.5 m/s; 30 s), and the combination of both treatments. The results demonstrated that biofilms formed on both copper materials produced lower contents of extracellular proteins and polysaccharides.

The reduction in biofilm culturability using copper materials was higher than the reduction caused by chlorine exposure or the mechanical treatment on biofilms formed on stainless steel. When the treatments were applied, lower number of CFU were detected in bulk water when copper materials were used. Chlorine depletion in plumbing systems is a common problem that favors biofilm regrowth. This work demonstrates that copper materials may be an important strategy to overcome this problem as their use had higher impact in biofilm control than the use of chlorine.

#### **P 114 Development and testing of low-cost solar photo-reactors for solar disinfection (SODIS) of harvested rainwater**

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Harvesting rainwater (HRW) is a common and valuable source of fresh water in rural areas of developing countries without access to centralized water supply systems. Nevertheless, it can be contaminated by pathogenic organisms and other chemical compounds (heavy metals and trace organic compounds) and an appropriate treatment is need to make HRW safe for drinking.

Solar disinfection (SODIS) is a household drinking water intervention treatment recognized by the World Health Organization. This technique is used by ca. 5 million people in developing countries on a daily basis. It consists on the exposition of 1-2L PET (polyethylene terephthalate) bottles to solar radiation under sunny conditions for 6 h. Nevertheless, the volume of water treated is one of the main drawbacks of this procedure. Several solar reactors based on the use of Compound Parabolic Collector (CPC) mirrors to enhance the efficacy of solar water disinfection have been investigated in the last decade, demonstrating to be a promising option, but still being an expensive option for low income areas.

The goal of this study was to develop and testing a new low-cost solar pilot scale reactor for treating harvested rainwater (HRW) using solar mirrors reducing the cost of manufacturing maximizing the efficiency of disinfection. Two pilot plants were built based on the new design but with different water volume capabilities (140 L and 90 L per batch). Solar disinfection of contaminated synthetic HRW with a consortium of waterborne pathogens has been assessed in both solar photo-reactors under natural sunlight. Results showed success inactivation efficiencies, obtaining > 5-log reduction on initial concentration of *E. coli*, *S. enteritidis*, *E. faecalis* and *P. aeruginosa* requiring a maximum solar UVA dose of 75 Wh/m<sup>2</sup> or 120 minutes of solar exposure.

#### **P 115 Off-grid UV-LED Water Disinfection – Surface Water Case Studies**

Babak Adeli and Ashkan Babaie

Acuva Technologies

Ingesting microbiologically contaminated water is one of the most common sickness routes for people living off the grid, such as remote communities and those travelling with recreational vehicles.

Ultraviolet (UV) radiation is known as the most effective water disinfection route. However, conventional UV-lamp based water purifiers cannot be utilized for off-grid applications, due to their high electrical power requirements and frequent maintenance. On the other hand, ultraviolet light emitting diodes (UV-LEDs) based water purifiers enable low energy consumption and maintenance-free operation.

Here, we will present the results of case studies on the off-grid disinfection of Grand River and Laurel Creek in Ontario, Canada as well as Fraser River in British Columbia, Canada, using UV-LED water purification system. The testing sites was selected based on the importance of the water resources to the community, as well as implemented restrictions due to microbial contaminations.

Grand River and Laurel Creek supply 30% of the Waterloo and Kitchener, Ontario drinking water. Yet, both are known to be heavily contaminated by Total and Fecal Coliform bacteria, being close to swimmers and tourists in the last few years. The onsite water disinfection tests were conducted using flowing and stagnant water, using Acuva's UV-LED water treatment system and carbon filter without pre-treatment. The bioassay tests were conducted in compliance to United State environmental protection agency (EPA) ultraviolet disinfection guidance manual (UVDGM), in collaboration with a third-party laboratory in Ontario, Canada. The microbial test data indicated that drinking safe water can be obtained using Acuva's UV-LED water treatment system, despite the low UV transmission expected for surface water. We will discuss that complete inactivation of Total Coliform is attributed to National sanitation foundation (NSF) 55 Class A ( $>40$  mJ/cm<sup>2</sup>) UV-dose delivery, which ensures water safety for off-grid applications, and can be realized via effective UV-LED reactor design.

**P 116 Household water treatment technologies for microbial removal In Kabale district, Southwestern Uganda**

Alex Saturday

Kabale University

**Background:** In Kabale District, health problems associated with the consumption of untreated drinking water is one of the greatest concerns inspite of government's efforts to provide safe drinking water to the people. This study, therefore, aims at assessing effectiveness of household water treatment technologies (HWT) and evaluate their ability to improve microbial quality of drinking water in Kabale District, Southwestern Uganda.

**Materials and Methods:** Five experiments were run to measure bacterial removal efficacy of HWT in addition to 205 respondents who participated in the study. Drinking water samples(from households) and source water samples into sterilized containers for and then transported to water quality laboratory for analysis. Membrane filtration method was used in analysis of water samples in accordance with APHA, (2005).

**Results:** Of 46 household treated water samples, 17.4% and 45.7% of water samples fell in no risk category (0 CFU/100 ml) for total coliforms and *Escherichia coli* respectively. Of 20 experiment treated water samples, 40% and 73% of samples fell in no risk category (0 CFU/100 ml) for total coliforms and *Escherichia coli*, respectively. Treatment by application of WaterGuard tablets achieved highest total coliforms removal with 99.5% (1.9 log10), whereas WaterGuard tablets, bio-sand filtration method, and aqua safe tablets achieved complete removal of *Escherichia coli* (100%) under laboratory conditions.

**Conclusion:** Spring water in Kabale District was found unsafe to drink unless treated. Effective water treatment products such as WaterGuard and aqua safe tablets should be promoted at local level. Local people should always be involved in simple household testing to reduce doubts on microbial efficiency of newly introduced HWT.

**P 117 Assessing the Impact of Water Treatments on Microbial Ecology in Pilot Drinking Water Distribution Systems**

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Although studies have focused on exploring the microbial ecology of DWDSs, knowledge about the effects of different water treatments on the microbial ecology in the distribution system is limited. This study was conducted at a drinking water production plant of the Oasen Water Company, the

Netherlands. The treatment plant uses well-confined, anoxic groundwater as its source water. After abstraction, the water was treated by aeration, filtration, softening, carry-over sand filtration, activated carbon filtration, and UV disinfection. This study assessed the impact of conventional treatment, RO treatment, remineralization, mixing water (70% RO permeate and 30% conventional water) on the microbial ecology in pilot distribution systems (PDSs). Results showed that remineralization has minor contribution to the biofilm formation with adding minerals into RO permeate, while 70% mixing reduce the biofilm formation, but much less than 70%. The bacterial community developed over a period of 10 months in all systems started from similar structures, but developed differently over time (Figure 3). The bacterial community of biofilms formed in RO system stayed the same over the whole period. Mixing of 70% RO permeate with 30% conventional water has no influence at all on the bacterial community. However, the option of remineralization showed significant influence on the bacterial community which formed a different cluster. A special focus was given to the *Legionella* and *Mycobacterium*. Results revealed that the growth of *Mycobacterium* spp. and *Legionella* spp. can be limited by RO permeate and remineralized RO permeated. In PDS supplied with Remin water, *Legionella* spp. was not detected, while *Mycobacterium* spp. was detected but with a much lower concentration comparing to that in feed and mix systems.

#### **P 118 Treatment of urban wastewater in High Rate Algal Ponds: factors affecting disinfection and aerosolisation of microalgae**

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Intensive microalgae cultivation systems such as High Rate Algal Ponds (HRAPs) constitute a promising way to remove macropollutants from urban wastewater. However, two sanitary issues are associated with this treatment process: the presence of pathogens that have to be inactivated and the potential aerosolisation of a high concentration of microalgae that could affect human health. Concerning pathogenic bacteria, factors affecting their inactivation in HRAPs are still not well characterized. Two pilot scale HRAPs operating under different climates (Oceanic and Mediterranean) were monitored to evaluate their capacity to remove fecal indicator bacteria (FIB) and genera containing opportunistic pathogens. Traditional plating techniques, qPCR and Illumina sequencing were combined to determine bacterial concentrations. In both HRAPs, average log<sub>10</sub> reduction values ranged between 1 and 2 Log for FIB. The clarification of wastewater and microalgal blooming induced a sharp decrease of the FIB and of genera containing opportunistic species (*Acinetobacter*, *Aeromonas*, *Arcobacter*, *Streptococcus*). At this time, important removal was recorded for culturable FIB (4.4 and 3 Log respectively, for thermotolerant coliforms and fecal streptococci), but not noticed when using qPCR. Differences between cultural and molecular results may be due to the presence of cells in a Viable But Non-Culturable State. After the blooming period, oxygen was the main factor affecting *E. coli* concentration, indicating that photooxidation may play a major role in the inactivation of this bacteria in HRAPs.

At the air water interface of these open water treatment processes, bioaerosols are emitted that could represent a health problem. In dedicated lab-scale instrumented aerosolisation tanks (1 m<sup>3</sup>), the capacities of microalgae from natural consortia to become airborne were compared. It was shown that cells of *Chlorella* are more prone to be aerosolised than cells of *Scenedesmus*-like species. The effect of water temperature, biomass concentration and the presence of surfactant on aerosolisation was evaluated.

**P 119 Impact of wastewater discharges on viral diversity and loads at a drinking water treatment plant**

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Raw drinking water supplies are commonly under the influence of (un)treated wastewater discharged upstream of drinking water treatment plants (DWTPs), a situation commonly described as de facto reuse. Data on the occurrence and the removal of enteric viruses by treatment processes during critical periods of contamination (rainfall and snowmelt events) are needed for exposure assessments. Prevalence and variation of seven viruses including rotavirus, adenovirus, astrovirus, norovirus, sapovirus, JC virus and enterovirus were assessed in an urban river in Quebec, Canada, during the snowmelt period of spring 2018.

Series of samples were collected to characterize raw and treated wastewater at an aerated pond discharging 5 km upstream a large DWTP. Additionally, at the DWTP, baseline and event-based samples were collected in raw, coagulated, ozonated, and UV disinfected water. Viruses were quantified using a combination of qPCR, cell culture and integrated cell culture with qPCR (ICC-qPCR). Serial 10-fold dilutions were performed for the cell culture on selected samples to calculate the original infectious virus concentrations by MPN method.

Rotavirus, adenovirus, and norovirus GII were the most common in raw wastewater with concentrations ranging from 4.3 to 6.2 log<sub>10</sub> genomic equivalent (GE) copies/L. Concentrations of rotavirus and adenovirus varying between 4.0 and 5.0 log<sub>10</sub> were measured in raw water during snowmelt events at the DWTP. The full-scale removal efficiency of these two viruses could be quantified for coagulation, ozonation, and UV-disinfection by concentrating volumes of 1000-2000 L. We are currently developing a statistical model to evaluate original infectious adenovirus concentrations. This case study indicates that only minor differences in viral loads could be observed between raw water at a DWTP and wastewater discharges in an urban river.

**P 120 SAFEWATER project– Addressing SDG6 in Latin America**

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The 2030 Sustainable Development Agenda agreed by the United Nations Member States in 2015 calls for universal access to safe drinking water, and the proposed indicator of 'safely managed drinking water services' will require measurement of drinking water quality. Therefore, the use of improved treatment and protection methods and management of drinking water sources for these vulnerable communities will become critical for achieving the new Sustainable Development Goal targets (UN, 2019). The lack of economic resources and water management strategies in low-to-middle-income areas and remote rural settings make the first option very difficult. Short and medium term, household water treatment and safe storage (HWTS) is a proven be cost-effective and cost-beneficial approach. HWTS can deliver significant savings that exceed the cost of delivery to health care systems, prevents waterborne diseases, significantly reduces childhood morbidity and mortality, considerably improves nutrition, productivity and school attendance, and results in better physical and cognitive development in these areas.

The GCRF-UKRI SAFEWATER project aims to develop low cost technologies to deliver safe drinking water in rural areas of Colombia and Mexico and develop associated devices that can evaluate the quality of drinking water in remote regions with no hi-tech laboratory access. These water technologies will be tested under real conditions, working with local, rural communities to evaluate health benefits that accrue after implementing the technologies.

This paper will discuss various HWTS that can effectively provide safe drinking water as determined by the WHO harmonized testing protocol for HWTS. According to this protocol, the technologies shall be evaluated for microbiological performance against four specific reference pathogens (*Escherichia. coli*, coliphage MS-2, bacteriophage phiX-174 and *Cryptosporidium parvum* infectious oocysts). The new SAFEWATER fast and easy low-cost technologies monitoring drinking water quality in rural communities in Colombia and Mexico will also be presented.

## P 121 Capability of ultra-filtration for total cell count reduction

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Ultra-filtration, which affects both chemical and microbiological processes, can be applied in a variety of ways in drinking water supply to improve water quality. Through the upstream addition of chemical agents or modification of water parameters the performance can be increased. According to the Austrian food law, disinfection of water has to be applied in a way that a 4-log-level reduction of pathogenic microorganisms is achieved.

In order to assess the efficiency of total cell count reduction by the application of a standard membrane with 0.02 µm pore size a pilot testing was carried out with two operating modes. Firstly, well water and secondly well water spiked with surface water was filtered. By using flow cytometry, in the time period in which well water was used as feed for the plant, values between roughly 2.4 and 3 log-levels were measured, whereas with the addition of surface water values up to 3.93 log-levels were attained. Based on the assumption that the raw water contains 250 000 cells per ml the amount in the measured 50 µl by flow cytometry would be 1.25 events inside the gate to reach 4 log-level-reduction of the total cell count. Due to possible false positive cell measurements originating from background signal or carryover combined with the possibility of cell growth in the filtrate tank and/or sampling tap, it is possible that the reduction is underestimated. For flow cytometry a lower detection limit is still up for discussion. Since there are only very few cells left in the filtrate the method can be used to assess the log-level reduction in terms of total cell count, in comparison to just consider pathogens which were never detected in the filtrate during pilot testing.

## P 122 Effect of microbial concentration on the disinfection performance of UV LED water treatment systems

Ashkan Babaie, Babak Adeli

Acuva Technologies Inc.

Growing concern over waterborne diseases is driving the demand for more efficient and effective water treatment systems. Drinking water can easily become contaminated with microbial pathogens such as bacteria, protozoa and viruses. Disinfection of water is a crucial part of the water treatment process. UV-LED water disinfection, takes advantage of all the proven benefits of Ultra Violet Technology in a more energy efficient way with additional benefits such as low maintenance and unlimited on-off cycling.

Using computer simulation and virtual prototyping for the design of UV-LED reactors is significantly beneficial in reducing the product development cost. Without the proper simulation tools, the reactor design may need to go through multiple design revisions and prototyping stages without delivering the

required UV dose or LRV at the end. Complete simulation of a reactor's performance requires precise modelling of reactor's hydrodynamics, optics and the microbial kinetics, each of which are equally important for an accurate reactor simulation. Using an in-house simulation software, we have managed to properly predict the dose delivery of the reactors which is consistent with the experimental bioassay tests.

Although the simulation tool is very capable of predicting the reactor performance in variety of different cases, it is noticed that at a certain combination of flow rate and high bacterial control concentration, the computer simulation and experimental results diverge significantly. It is believed that shear-dependent aggregation of bacterial strains such as e-coli is responsible for introducing new physics which is classically not considered in computer modellings.

In this study, using mathematical modelling supported with experimental observation we explain how performance of UV water disinfection systems can be different under high bacterial control concentrations and low flow rates due to bacterial aggregation; whereas, using same reactor under same flow rate, a different UV dose delivery can be expected for virus disinfection where microbial aggregation is not expected.

**P 123      Antibiotic-resistance phenotypes and UV resistance among ESBL-producing *E. coli* isolates from WWTP effluent in Hong Kong**

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Global integrated surveillance of the dissemination of extended spectrum  $\beta$ -lactamase-producing *E. coli* using “One Health” approach is proposed by World Health Organisation. The purpose of this study is to investigate resistance patterns and DNA fingerprints of these ubiquitous microorganisms from the effluent of the wastewater treatment plant in Hong Kong and minimize its spread in the environment. Phenotypic confirmation of ESBL-producing *E. coli* was performed according to EUCAST guidelines for disk diffusion test in agar plates. Isolates were also tested against seven other antibiotics, upon which, co-resistance with ceftazidime or cefotaxime, in clinical settings, is observed or monitored. Antibiotics tested, for this purpose, are: sulphamethoxazole/trimethoprim, sulphamethoxazole alone, ciprofloxacin, amikacin, gentamicin, colistin sulphate and imipenem. Apart from the 3rd generation cephalosporins, most isolates were also resistant to ciprofloxacin (18/21) and co-trimoxazole (15/21). These resistance patterns are in accordance with local hospital reports, according to which, ESBL-producing *E. coli* showed high resistance toward non  $\beta$ -lactam antibiotics, such as fluoroquinolones, co-trimoxazole and aminoglycosides. Further characterization of the isolates was done, by visualization of the plasmid DNA on agarose gel. To minimize microbial spread in the environment, conventional, mercury-vapour, UV lamps are commonly employed to disinfect wastewater effluents after traditional treatment processes. Since their disposal is a significant environmental issue, UV-LEDs, are emerging as a sustainable alternative. In this work, we aimed to address certain contradictions about selective microbial responses to UV disinfection. We have investigated UV susceptibility of three ESBL-producing *E. coli* strains, with different DNA characteristics, and a type-strain (ATCC 25922) upon disinfection with UV-LEDs at 3 different wavelengths (265, 285 and 300 nm). Our current results suggest that, when bacteria are exposed to sublethal doses of UV light, selection of clinically relevant, antibiotic resistant bacteria may occur, resulting in an increased proportion of ESBL-producers in the population released after the treatment.

**P 124      Effect of Inorganics on the Microbial Disinfection Performance of UV Water Treatment systems**

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Inorganic minerals in water are classified into "healthy" and "unhealthy" compounds. Primary water treatment technologies, such as membranes and advanced filtration, has placed an emphasis on the elimination of unhealthy minerals such as lead, arsenic, and antimony; while preserving those benefit human health, such as calcium, magnesium and potassium. In particular, calcium and magnesium are the most common minerals in water supply, and their concentration is recognized as the water hardness indicator.

Despite their health benefits, minerals tend to deposit inside disinfection systems, particularly, in the vicinity of conventional UV lamps, owing to their temperature dependent solubility in water. In addition, hard water exhibits lower UV transmittance (UVT), compared to water with less than 120 ppm minerals content, the so called "moderately hard" water. Therefore, the disinfection performance of UV water treatment system is a strong factor of water hardness level, and the application of UV-based water disinfection systems is limited in the regions with high mineral content in water supply.

Here, we will discuss the effect of water inorganics level on the performance and reliability of UV disinfection products. UV-LED and UV-lamp disinfection systems were operated with water contains various levels of inorganics, and their optical characteristics were evaluated. Furthermore, the effect of mineral and ions on water UVT, and subsequently the microbial disinfection performance of UV systems was correlated. We will propose several guidelines for operation of UV systems for regions with high mineral content water. It is shown that UV-LED disinfection systems exhibit consistent microbial disinfection, owing to uniform temperature gradient across the reactor; however, scaling of inorganics inside the disinfection chamber reduces the disinfection efficacy of UV-reflective chambers. The outcome of this study provides an insight on reliable operation of UV disinfection systems.

**P 125      Efficient Step-feed Partial Nitrification, simultaneous Anammox and Denitrification (SPNAD)**

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The efficient step-feed partial nitrification, simultaneous Anammox and denitrification (SPNAD) equipped with real-time control parameters, an innovative approach, was feasible to achieve nitrogen removal from raw mature landfill leachate. The process had achieved advanced and enhanced nitrogen removal by three periods of consecutive aerobic/anoxic phases and six steps of feeding in every operational cycle. Oxidation reduction potential (ORP) and pH were utilized as real time on-line parameters (Fig. 1). In aerobic phases, aeration should be stopped the moment break point ("Ammonia Valley") appeared in pH curve. In anoxic phases, the set point ("Nitrate Knee" and "Nitrate Apex") emerge in ORP and pH profile, it is time to end the anoxic phases and restart aeration. The nitrogen removal efficiency (NRE) of 98.7% and nitrogen removal rate (NRR) of 0.23 kg m<sup>-3</sup>d<sup>-1</sup> were obtained at the influent ammonia, SCOD and total nitrogen (TN) of 1000±250 mg L<sup>-1</sup>, 1100±200mg L<sup>-1</sup>, and 1300±75mg L<sup>-1</sup>, respectively. In each anoxic phase, nitrate produced by Anammox was firstly reduced to nitrite by denitrifiers, which would be also served as substrate for Anammox. Mass balance research demonstrated the average nitrogen removal contribution by Anammox were 12.2% in aerobic phases and 69.3% in anoxic phases.

The underlying mechanisms were investigated by using Illumina MiSeq sequencing to analyze the 16S rRNA gene of bacteria. A remarkable Anammox community structure changing (Ca. Brocadia from 0.26% to 2.13%, Ca. Kuenenia from 0.29% to 0.02%) mainly attributed to different kinetic strategies (R-strategist of Ca. Brocadia and K-strategist of Ca. Kuenenia), and further study indicated co-existence of functional microorganisms Nitrosomonas (3.0%), Cadidatus-Brocadia (2.13%), and Thauera (25.3%) (Fig. 2).

## P 126 Inactivation of *Cladosporium sphaerospermum* by catalytic ozonation

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*Cladosporium* is a fungus widely present in drinking water, food and living environment. *Cladosporium* is known as a black mold which grows on materials and food. *Cladosporium* is an important allergenic fungus worldwide. *Cladosporium* is a disinfectant-resistant microorganism. A better inactivation method of *Cladosporium* is necessary to maintain life and water sanitation. Catalytic ozonation is an advanced oxidation treatment method using hydroxyl radical generated by adding a solid catalyst to ozone. Although there have been some studies on inactivation of *Cladosporium* by ozone, there has been no report on the inactivation of *Cladosporium* by the catalytic ozonation. In this study, inactivation of *Cladosporium* was studied by ozonation, advanced oxidation using ozone/hydrogen peroxide, and catalytic ozonation using ozone/zinc oxide or ozone/titanium dioxide. In all experiments except for controls, the initial ozone concentration was set at 1.5 mg/L and the suspension of *Cladosporium sphaerospermum* was disinfected in an open batch-type reactor. In order to investigate the influence of hydroxyl radicals, tert-butyl alcohol was also added to the reactor. In the advanced oxidation using ozone/hydrogen peroxide and the catalytic ozonation using ozone/zinc oxide, the initial inactivation rate of *Cladosporium* was higher than the ozonation. In catalytic ozonation using ozone/titanium dioxide, the initial inactivation rate of *Cladosporium* was about the same as that in the ozonation. When tert-butyl alcohol was added as a radical scavenger to the advanced oxidation treatment reactor using ozone/hydrogen peroxide and the catalytic ozonation using ozone/zinc oxide, the initial inactivation rate of *Cladosporium* was about the same as that in the ozonation. It was suggested that the increase of the initial inactivation rate in advanced oxidation treatment using ozone/hydrogen peroxide and by the catalytic ozonation using ozone/zinc oxide were due to the action of hydroxyl radical.

## P 127 Feasibility and Programmatic Comparison of Poliovirus Environmental Surveillance Methods

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Environmental surveillance (ES) of poliovirus is crucial for understanding its circulation as eradication nears. The Global Polio Eradication Initiative, led by the World Health Organization (WHO), characterizes if ES methods are acceptable, with each method suited to different use cases. An acceptable ES method must demonstrate that PV detection is as good or better than the WHO-recommended method, aqueous polymer two-phase separation (Two-Phase), and must also consider timing, cost, and procurement. The bag-mediated filtration system version 2 (BMFSv2) is a WHO-acceptable ES method, which yields enhanced surveillance sensitivity via in-field filtration of large sample volumes (up to 6-L wastewater) and secondary concentration to a final 10-mL volume. This study compared BMFSv2 with Two-Phase, based on poliovirus recovery, timing, cost, and procurement considerations. Samples were prepared by seeding ~104 PFU poliovirus type 1 (PV1) into 0.5-L primary influent wastewater (Two-Phase) and 6-L primary influent wastewater (BMFSv2).

Two-phase samples were centrifuged (4°C, 1500G, 20 minutes) to separate out solids. The liquid fraction was combined with polyethylene glycol and dextran, added to a separatory funnel, and held overnight at 4°C. The lower hydrophilic layer was retained and organic solvent extracted. BMFSv2 samples were filtered using 2" ViroCapTM filters followed by two elutions with a beef extract glycine solution, secondary concentration via skimmed milk flocculation, and organic solvent extraction. Final sample concentrates were assayed via BGMK plaque assay. PV1 recoveries were determined to be as good or better when using BMFSv2 (37%, n=11) than when using Two-Phase (4.3%, n=10). Laboratory timing and cost estimates were comparable between the two methods, while the BMFS required more time in the field. Finally, all BMFSv2 components are currently commercially available, and commercialization of the overall kit is ongoing. This demonstrates that BMFSv2 is an acceptable option for poliovirus ES, and should be utilized for specific use cases.

**P 128 Feasibility of electrochemical oxidation as a disinfection treatment of food wash water**

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Fruits and fresh vegetables can be vehicles of transmission for all kinds of pathogenic microorganisms, especially when eaten raw. To reduce the health risk associated to their consumption, the processing industry wash these foods adding sanitizers such as chlorine before packaging and distribution. In this work we study the feasibility of the electrochemical oxidation technologies to reduce the presence of foodborne pathogens.

Electrochemical oxidation technologies are beginning to be applied as disinfection treatments of wastewater, ballast water and as well in swimming pools. In this work we study the capability of using electrochemical oxidation treatment to reduce the presence of different types of microorganisms in the food wash water. Many operational parameters effect the disinfection treatment efficacy of electrochemical oxidation, three of them are very important: the type of electrodes, the type of electrolyte, in this case the wash water characteristics and the type of microorganisms. Here we chose dimensional stable anodes (DSA) because its capacity of electro generating species of chlorine along with hydroxyl radicals, and as wash water we used water from a phreatic of Barcelona city highly mineralized. Before its use as electrolyte, the water was decalcifier to reduce the presence of divalent ions. To test the electro disinfection capacity the wash water was doped with Escherichia coli, MS2 bacteriophage and spores of *Clostridium perfringens* as indicator microorganisms. The results indicated that the electrooxidation of the phreatic water with DSA electrodes is efficient to reduce the pathogens from the food wash water and so, it could be a good technique to eliminate these microorganisms of fruits and vegetables.

**P 129 UV-LED, peroxide chemicals and ozone disinfection in different water matrixes**

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In this study, UV light, UV-LED light, peroxide chemicals and ozone were tested separately and with combinations of techniques to deactivate test microbes in different water matrixes. The disinfection experiments were made for drinking water and treated wastewater.

In the disinfection experiments, the efficiency of traditional mercury lamp UV light and the new UV-led technology were compared. Both UV techniques were combined with peroxide chemicals (PAA, H<sub>2</sub>O<sub>2</sub>), ozone was tested separately. The pilot scale test system consisted of 250 L plastic container, pump, pipelines, rotameter, disinfection devices and 30 L sample tanks. Bank filtrated and chemically treated, undisinfected water from Jänneniemi waterworks, Kuopio, Finland was used as drinking

water. Inoculated test microbes were *E. coli* WDCM 00012, MS2 coliphage NCTC 12487 and *C. bifermentans* NCTC 506. In experiments with waste water effluent, the removal of naturally occurring *E. coli* and coliform bacteria, F- specific and somatic coliphages and the spores of sulphite-reducing clostridia were analyzed. Wastewater was taken from the Kuopio city's wastewater treatment plant Lehtoniemi.

In general, *E. coli* was more susceptible to inactivation than the MS2 coliphages or spores of *C. bifermentans*. Preliminary results show that *E. coli* numbers in drinking water and waste water decrease when peroxide chemicals were used in combination with the UV or LED-UV light than when using UV-light alone. Ozone was shown to be effective disinfectant. It seems that combination of techniques may result higher reduction results in water disinfection than using these techniques alone and therefore it can be profitable to use them together.

#### **P 130      Resistance of Indigenous *E. coli* and *Enterococcus* spp. in Wastewater to Disinfection**

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While much of the data in the bacteria disinfection literature was determined using bacteria grown in the laboratory as challenge organisms (i.e., environmental isolates or laboratory-strain bacteria), we have observed indigenous, wastewater-sourced bacteria to be more resistant to disinfection than their laboratory-grown counterparts. Experiments were conducted to evaluate and model disinfection kinetics of laboratory-grown and wastewater-sourced *E. coli* and *Enterococcus* spp. with exposure to chlorine and simulated sunlight. The experiments utilized washed and concentrated bacteria inoculated into PBS; laboratory and wastewater bacteria were prepared in the same manner, indicating that differences in disinfection rates were not due to the purification procedure. *E. coli* and enterococci indigenous to wastewater were more resistant to chlorine disinfection and sunlight inactivation than *E. coli*, *E. faecalis* and *E. faecium* cultured in the laboratory. For example, observed first-order decay rates of laboratory-grown bacteria were approximately ten times faster than those for wastewater-sourced bacteria. Control samples for the chlorine disinfection experiments found minimal presence of aggregates in wastewater bacteria inoculums (as measured by plate counts before and after filtration with a 10- $\mu\text{m}$  Durapore filters). Additionally, filtration with 10- $\mu\text{m}$  pore size filters prior to experiments did not significantly impact chlorine disinfection rates, signaling that the apparent resistance of wastewater bacteria to disinfection was not due to the presence of bacterial aggregates. These findings raise questions surrounding the application of laboratory-derived data in disinfection practice. Ongoing research utilizing culture-based and molecular assays is being conducted to determine the extent and mechanisms of bacterial resistance to sunlight, chlorine, chloramines, and ultraviolet radiation. bacterial resistance to sunlight, chlorine, chloramines, and ultraviolet radiation.

#### **P 131      Evaluating Water Safety Plans in some Pilot Communities in Northern Ghana**

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Water Safety Plans (WSP) requires preventive risk management that includes regular monitoring of drinking-water quality. A pilot baseline and endline study of the water quality of selected sources of drinking water in some selected communities (Water Safety Plan communities) and control communities (that is those without Water Safety Plan systems) were conducted in three regions in the Northern part of Ghana. Samples obtained from 150 labelled community taps or sources were analysed for Residual Chlorine, Total Coliform, Faecal Coliform and *Escherichia coli* counts during baseline and endline studies. Interventions involving health education of WSP managers and the general community were conducted after baseline studies. Managers of the community water supply system were also trained before the program commenced. Baseline results show that no sample had

residual chlorine concentration conforming to the Ghana Standards Authority recommended minimum value of 0.2mg/l and Coliform levels above WHO recommended levels were observed at 109 collection points (72%). Endline results show that taps that had low pumping pressure and were running intermittently, had high coliform counts. Endline results show that forty-eight water samples (32%) had significant ( $\geq 20\text{cfu}/100\text{mL}$ ) coliform contamination and seventeen (17) water samples (11.3%) had significant ( $\geq 20\text{cfu}/100\text{mL}$ ) faecal coliform contamination. Dosing with the appropriate concentration of chlorine seems to be a challenge for the managers of the Water Safety Plan in the pilot communities. Excreta of farm animals were commonly seen in the communities, including residential compounds and this may be a key source of contamination of domestic water storage facilities. Recommendations for improved management are discussed.

**P 132      Understanding Microbial Loads in Wastewater Treatment Works as Source Water for Water Reuse**

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Reuse of treated municipal wastewater has the potential to relieve the ever-increasing demands of providing water for (non-)potable use. However, negative public perception and health concerns due to the uncertain microbial quality of reclaimed water limit its widespread use and applications. Thus, additional research is needed to further characterize the microbial pathogens of concern present in wastewater effluent and the risks associated with exposure to these contaminants. The objectives of this study were to assess microbial quality of reclaimed water and to investigate treatability of microorganisms during different treatment trains. Wastewater samples from three participating utilities were collected monthly for one year. Fecal indicators like, male-specific coliphages, somatic coliphages, total coliforms, fecal coliforms, *Escherichia coli*, and aerobic endospores were measured as well as concentrations of *Cryptosporidium*, *Giardia* and infectious human adenovirus were monitored in raw and final treated effluents. Results revealed that human adenovirus and *Giardia* cysts were detected in 98% (46/47) of influent samples, whereas 66% (31/47) of the samples were positive for *Cryptosporidium* oocysts. In treated effluent samples, the prevalence of infectious adenovirus markedly decreased to 60%. Averages of Log10 reduction of adenovirus between influent and effluent for all three utilities ranged from 3.52 to 4.13, whereas relatively lower Log10 reductions with a range from 1.28 to 3.04 were achieved for protozoan parasites. Log10 reduction of bacteriophages were greater than that of human adenovirus, suggesting that bacteriophages analyzed could be considered conservative viral indicators of human adenovirus. Overall, this study will assist municipalities considering wastewater effluent as another source of drinking water important data on the prevalence, occurrence, and persistence of waterborne pathogens of concern. More importantly, the results from this study will aid in building a richer microbial occurrence database that can be used towards revising reuse guidelines and disinfection practices for water reuse practices.

**P 133      Impact of climate change on agricultural practices: reuse of municipal reclaimed water and treated greywater and respective implications for crop irrigation and human health**

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Water is the primary vehicle through which the effects of climate change will be felt with access to water becoming less predictable. Droughts are aggravating profoundly water scarcity throughout the globe, affecting its quality, productivity with increased risks to humans. This problem deepens further as potable water is still being used worldwide for crop irrigation. In this work, secondary and chlorine disinfected wastewater and greywater were used for irrigation of crops to determine their potential use

in agricultural practices. Tomato, lettuce and carrot seeds were planted and watered with these waters outside the laboratory facilities, mimicking real-life agricultural practices as best as possible. Irrigation waters and produce were analyzed for the three main classes: bacteria (*Escherichia coli*, intestinal enterococci, *Salmonella* spp. and *Pseudomonas* spp.), enteric viruses (Norovirus GI and GII, Enterovirus and Aichi virus) and protozoan (*Cryptosporidium* spp. and *Giardia* spp.). The percentage of positivity in greywaters was 100% for intestinal enterococci, 91% for *Escherichia coli* and 45% for *Pseudomonas aeruginosa*. *Salmonella* was negative in all greywaters and in the two treated wastewater samples. The percentage of removal for bacteria during the wastewater treatment plant varied between 99% for *Escherichia coli* and *Pseudomonas aeruginosa* and 100% for intestinal enterococci and *Salmonella*. Conversely, the removal for enteric viruses ranged between 58% for Norovirus GI and 87% for Enterovirus. Preliminary results showed the presence of Norovirus GI in the interior of carrots and of Norovirus GI and Enterovirus in the tomatoes irrigated, both irrigated with disinfected wastewater. This study highlights the need for further knowledge of the impacts in human health associated with the consumption of fresh produce irrigated with these waters.

#### **P 134      Growth of Bacterial Population in Reclaimed Greywater Distribution Systems of the Pilot-Scale Membrane Bioreactor**

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With advances in wastewater treatment technologies, the reuse potentials of reclaimed water are expanding. Some citizens are concerned about the safety of using reclaimed water, particularly with respect to health risks associated with the growth of the bacterial population found in the distribution systems. In this study, the reclaimed effluent from reclamation treatment was simulated to evaluate the impact of reclaimed effluent on the growth of the bacterial population inside the distribution networks. A pilot distribution system of the membrane bioreactor system (MBR) treating of household grey water was used to perform these experiments. An attached biofilm on media within the reclaimed wastewater distribution device of the effluent was identified and evaluated bacterial regrowth population in association with the types of organic matter components. A special focus of reclaimed effluent was set on the study of the selection of organic micropollutants from household chemicals (e.g. surfactant plasticizer and biocide). Besides, the water quality of outflow effected in the pilot unit has been also evaluated. The results revealed that the bacterial growth composition on attached biofilm remarkably changed when compared to suspended sludge in MBR. The relative abundance of Sphingomonadales order which belonged to Alphaproteobacteria was predominantly observed on attached biofilm. Moreover, the proportion of Burkholderiales order belonged to Betaproteobacteria, particularly *Methylibium* sp. on attached biofilm was much higher than that in suspended sludge. The spectroscopic analysis identified the humic and/or fulvic acid-like substances and protein-like substances as the main components in the effluent MBR. The formation of organic matters might be related to bacterial growth population. Based on the results, it is suggested that an appropriate post-treatment is needed to maintain the biological stability of reclaimed wastewater

#### **P 135      Evaluation of viruses common to wastewater as indicators of reverse osmosis membrane system performance for potable reuse applications**

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University of Arizona

Advanced wastewater treatment processes are indispensable for mitigation of microbial and chemical contaminants within potable wastewater reuse systems. Integrated membrane systems, micro or ultra-filtration coupled with reverse osmosis (RO), are often implemented in advanced treatment processes due to their ability to remove molecular-sized contaminants, down to the ionic level. Viruses are of

particular relevance in potable wastewater reuse schemes due to their acute health effects, low infectious dose, small size, and resistance to disinfection. Currently, studies on virus occurrence after RO membrane processes in potable reuse treatment systems are scarce. The goal of this project was to investigate multiple indigenous virus targets as indicators of treatment performance of integrated RO systems from pilot and full-scale advanced treatment plants in Arizona.

Viruses were concentrated from treated wastewater effluent ( $\leq 100$  L) and RO permeate (1000+ L) using adsorption-elution methods followed by centrifugal ultrafiltration. Viruses were detected by quantitative polymerase chain reaction assays targeting viruses frequently recovered from wastewater effluent at values between 102 to 107 genome copies (gc) per liter, on average, including: human adenoviruses, two plant viruses (pepper mild mottle virus [PMMoV], cucumber green mottle mosaic virus [CGMMV]) human bocavirus (HBoV), and a newly discovered circular Rep-encoding single-stranded (CRESS) DNA virus (WCDV-2). Our results indicate the intermittent detection of PMMoV, CGMMV, and WCDV-2 in RO permeate at values between 5 and 50 gc per 100 L. The presence of these viruses in RO permeate streams highlights the potential application of multiple viruses indigenous to wastewater as indicators of RO membrane integrity in full-scale advanced wastewater treatment systems for potable reuse.

**P 136 Molecular identification of *Giardia duodenalis* cysts in reuse water from two sewage treatment plants in São Paulo, Brazil**

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Water reuse is a reality in many countries because of the economic and environmental benefits that water recycling provide, however, the parasitological quality of such water must be monitored to protect the population's health. In Brazil, there is legislation that establishes quality parameters for the use of this resource only at some states, including São Paulo. The presence of *Giardia duodenalis* in recycled water is a risk factor for the exposed population because it is the main species that causes disease in humans. The aim of this work was to identify *Giardia duodenalis* in recycled water from sewage treatment plants. Using the USEPA protocol 1693/2014, the samples (30L) were collected and processed monthly from two wastewater treatment plants (WWTP) in São Paulo (A and B) between 2016 and 2017. Results revealed the presence of *Giardia* cysts in 36.5% of the samples analyzed, with concentrations ranging from lower than detection limit (LD  $< 0.03$  cyst / L) to a maximum of 16 cysts / L. The slides were scraped and the cysts recovered, and the DNA was extracted for the characterization of the cysts by qPCR. The molecular identification was performed by the presence of the 18S SSU gene for *Giardia duodenalis*. In 29.6% (8/27) of WWTP "A" samples, *Giardia* cysts were present, and 11.11% (3/27) contained the 18S SSU gene from *Giardia duodenalis*. For WWTP "B", in 44% (11/25) of the samples *Giardia* cysts were found, of which 12% (3/25) contained the 18S SSU gene from *Giardia duodenalis*. The results indicate the presence of *Giardia duodenalis* in reuse water, which may pose a risk to human health as these waters are discharged in urban environment or destined to other usages that could pose a health risk for exposed population, especially elderly, children and other immunocompromised individuals.

**P 137 Coupling water quality modelling with quantitative risk assessment for consumers of the uMsunduzi River, South Africa**

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In South Africa, water quality challenges arise mainly from anthropogenic activities. These include chemical waste from industries, metals from mines, excessive nutrients, phosphates and coliforms from wastewater treatment networks, and salts and other toxic substances from agricultural activities.

A large proportion of the sewage emanating from South African urban areas is not treated properly prior to discharge. This is due to dilapidated and overloaded sewer systems. This study will focus on uMsunduzi catchment in Pietermaritzburg, KwaZulu-Natal, South Africa. UMsunduzi River is a major tributary of uMngeni River, which flows into Inanda Dam - Durban's primary water supply. It has been found that the water quality monitoring results of uMsunduzi confirm poor to very poor levels of water quality. Recent studies have revealed microbial contamination with organisms associated with waste disposal such as *Salmonella spp.*, *enterococci*, and *E.coli*. This renders uMsunduzi River, unsuitable for human consumption without treatment, and largely unsuitable for recreational use. The recreational use of uMsunduzi River ranges from big events such as the Duzi Canoe Marathon which takes place in mid-summer, to small-scale fishing, to small children swimming in the river to pass time. Rural and informal settlement occupants use the river water for laundry, amongst other chores. A microbial water quality model that represents uMsunduzi catchment was setup within ArcSWAT2012. The model was calibrated and validated using available data. This model forms a foundation for a water management decision-support system that looks into health risk assessment to the river users for recreational and domestic uses. The microbial output from the model is the input in Quantitative Microbial Risk Assessment (QMRA) for specified users. The risk data can be used by catchment managers to prioritise areas of high risk within the catchment. Different scenarios can be input to predict the impact of different scenarios on the end-users.

### **P 138 Effects of sediment on fate and transport of faecal bacteria in Dublin Bay**

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Faecal indicator bacteria (FIB) exists in both free-living and attached form in source and receiving waters. The fate and transport of faecal bacteria are affected by many environmental, chemical and biological factors. The fate of bacteria also varies depending on whether the FIB reside in waters with high concentrations of suspended sediment or are resident in bed sediment. An increasing number of studies have shown that faecal bacterial levels in sediments can be high relative to those in water columns. Recently conducted research has revealed the presence of high faecal contamination in the nearshore sediments of Dublin Bay, presenting a risk that these bacteria may release into the associated bathing waters with the potential that bathing water quality may be adversely affected. However, the transfer of this faecal bacteria in bed sediments to bathing waters in Dublin Bay has yet to be quantified and this is the focus of the study. The paper presents the development and application to Dublin Bay of a 3-dimensional numerical model with capacity for simulating the effects of sediment transport processes on the fate and transport of faecal bacteria. The hydrodynamic characteristics of the model have been validated with high resolution measurements of current speed and direction from within the model domain, faecal indicator bacteria concentrations (*E.coli* and Intestinal Enterococci) in bed material determined from sediment grab samples and from faecal indicator bacterial in the bathing waters. The work presented forms part of the EU INTERREG funded Acclimatize project ([www.acclimatize.eu/](http://www.acclimatize.eu/)) that is investigating the longer-term water quality pressures in Dublin Bay that may arise in the context of a changing climate.

**P 139 Modelling the risk to contamination to private water supplies in Scotland**

Sughayshinie Samba Sibam

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Private water supplies have been identified as a risk factor for gastrointestinal illness in Scotland. The vast majority of private water supplies are small scale supplies, which number approximately 20,000. The responsibility for managing these supplies resides solely with their owners and users. As a consequence, the quality of water from these supplies can often go untested for years. The types of private water supplies can vary greatly but are generally grouped into either surface water fed or groundwater sourced, which influences how vulnerable the supply is to contamination.

Diffuse pollution by organisms such as *Cryptosporidium*, *Escherichia coli*, *Salmonella* and *Campylobacter* is a major threat to the quality of these supplies with potential public health consequences for waterborne related illness. Some component of this contamination is dependent on sporadic environmental conditions such as adverse weather events and temporal land use. The impact on the vulnerability of the supply from these events is dependent on associated factors, such as the density of livestock within close proximity. In addition, the faecal output and the prevalence of pathogens varies between types of animal.

Building on evidence related to the various risk factors contributing to the microbiological contamination of private water supplies, we develop a mathematical model that explores the transmission of pathogenic organisms from the sources to the supplies. This analysis is applied within our three study areas with high private water supply use. This model will assist in the prioritisation of testing of small scale supplies and will highlight potential hotspots for risk to microbiological contamination.

**P 140 Predictive Water Virology: Hierarchical Bayesian Modelling for Estimating Virus Inactivation Efficiency**

Daisuke Sano, Syun-suke Kadoya

Tohoku University

The hazard analysis and critical control point (HACCP) approach is a series of actions to be taken to ensure safety in the product consumption. Water Safety Plans (WSP) and Sanitation Safety Plans (SSP) are based on the principles of HACCP for the safe utilization of water and reclaimed wastewater, in which the operational monitoring at the critical control points is a key action component. In the food poisoning risk management, the predictive microbiology is a field of study to provide the minimum stress (such as temperature and contact time in the heat treatment) for the sufficient microbe inactivation based on mathematical models. However, the application of the predictive microbiology to pathogens in water is difficult because the complex composition of water matrix does not allow us to make a simple mathematical model to predict the pathogen inactivation efficiency. The objective of this study was to construct the disinfection model for water-related viruses according to the various water qualities in water treatment plants considering the uncertainty among multiple studies and the difference in the genotype-dependent sensitivity to disinfectants. Data of virus inactivation efficiency by free chlorine were collected according to the framework of systematic review using Google scholar. The hierarchical Bayesian modeling was adopted using R and Stan codes to take the uncertainty among researches and difference in free chlorine sensitivity among virus genotypes into account. As a result, we successfully conducted hierarchical Bayesian modeling of the EFH model parameters for norovirus, rotavirus, hepatitis A virus, coxsackievirus, echovirus and adenovirus. These models must contribute to the determination of critical control points in WSP and SSP to ensure the safety in water usage.

## **P 141 Bathing Water Quality Modelling in Dublin Bay**

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University College Dublin, School of Civil Engineering (A), University College Dublin School of Biomolecular and Biomedical Science (B), University College Dublin School of Computer Science (C)

This paper presents the development and preliminary results (establishment of baseline) of a deterministic modelling system for bathing water quality assessment in Dublin Bay, Ireland. The system integrates functional capacity for simulating the transport and fate of diffuse agricultural pollutants (utilising both the NAM rainfall-runoff model in conjunction with MIKE 11), discharges from the Dublin urban drainage network (through MIKE Urban and InfoWorks software), and the ultimate fate of pollutants in Dublin Bay (the coastal domain modelling utilising the 3-dimensional MIKE 3 code). The work presented forms part of the EU INTERREG funded Acclimatize project ([www.acclimatize.eu](http://www.acclimatize.eu)) that is investigating the longer-term water quality pressures in Dublin Bay that may arise in the context of a changing climate (particularly that from predicted changes in precipitation totals and patterns). Model calibration and validation has been underpinned by extensive data collection from within the catchments discharging to Dublin Bay and from the bay area itself. Catchment data includes the observing of hydrometeorological variables for establishing relationships to measured flows and water quality at catchment and sub-catchment scales. Coastal data relates to water quality, coastal hydrodynamics (current speed and direction collected from ADCP deployments at multiple monitoring points in the bay), temperature and salinity. A nested modelling approach where the modelled domain is nested in a larger Irish Sea model has been adopted. Tidal constituents along the seaward boundaries of this nested model have been calibrated to correlate well with tidal measurements from a set of established tide gauges within the modelled domain. Bottom friction was calibrated to produce good correlations of measured and simulated current speed and direction. Preliminary results indicate that the transport of faecal indicator bacteria within the study area is adequately represented for spring and neap tide conditions.

## **P 142 Mapping sanitation-associated pathogen contamination of surface waters in Uganda**

Daniel A. Okaali, Carolien Kroeze, Gertjan Medema and Nynke Hofstra

Wageningen University and Research

Pathogen pollution of water sources is frequently linked to poor sanitation systems management. Such pathogens are responsible for their associated pervasive disease burden, particularly in developing countries, and are primarily transmitted faecal-orally through ingestion of contaminated water and food. Open defecation, inadequate or non-treatment of human waste are cardinal pathways through which pathogens reach water sources. System-wide pathogen modelling and mapping can supplement quality monitoring in data-sparse areas. We, therefore, present the Pathogen Mapping Tool, which estimates pathogens loads and concentrations in surface water from onsite and offsite sanitation systems in Uganda. This tool integrates population density, onsite and offsite sanitation coverage, pathogen incidence, treatment removal and transport in water systems to produce spatio-temporally explicit surface water pathogen levels. We also present future scenarios on the impacts of sanitation management, socioeconomic development and climate change on modelled pathogen loads and concentrations. One of the tool's models estimating rotavirus and *E. coli* loads from offsite sanitation and open defecation, was previously applied to Uganda for contemporary conditions in 2015 and for scenarios in 2030. It revealed densely populated areas like Kampala as key hotspots, while urban open defecation and non-treatment were the largest contributors. In the scenarios, improving faecal waste treatment, reducing non-treatment and ending open defecation were critical in decreasing microbial loads to surface water.

The results for Kampala, incorporating onsite sanitation, revealed a 1-log addition to total loads, necessitating the integration of onsite systems into the concentrations model. Our tool will identify key sanitation pathogen sources, hotspots and their relative spread across study areas. From scenarios, understanding the impact of sanitation management interventions on pathogen concentrations in water systems will allow practitioners make sound decisions to improve microbial water quality. Finally, we also plan to use the tool's pathogen concentrations output as input for risk assessment.

#### P 144 Future *Cryptosporidium* concentrations in rivers worldwide

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*Cryptosporidium* still is one of the main pathogens that cause diarrhoea, in particular in children younger than 5 years old. Water is a main pathway through which *Cryptosporidium* is spread. *Cryptosporidium* concentrations caused by poor waste management of human faeces could change in the future through, among others, population growth, urbanisation, changes to waste management and climate change. However, future changes to *Cryptosporidium* concentrations have not been studied before. We present simulated present and future *Cryptosporidium* concentrations in rivers worldwide. The Global Waterborne Pathogen (GloWPa) model has been applied for human faecal waste and simulates spatially-explicit *Cryptosporidium* concentrations in rivers worldwide. Human *Cryptosporidium* emissions and concentrations to surface water had been simulated before. We use two scenarios to estimate changes in the concentrations for approximately the year 2050. Scenario 1 combines the Shared Socioeconomic Pathway (SSP) 1 and Representative Concentration Pathway (RCP) 2.6 of the Intergovernmental Panel on Climate Change. In this scenario the world sees moderate population growth, equality, transfer of knowledge and technology across the world and there is a strong focus on the environment. The temperature increase is limited to around 2oC. Scenario 2 combines SSP 5 and RCP 8.5. In this scenario there is strong economic growth, rapid technology development and the environment is not considered to be important. The temperature increase in this scenario is around 5oC. Changes in sanitation and waste water treatment have been developed from the scenario narratives and are related to the GDP. The *Cryptosporidium* concentrations are reduced for both future scenarios. We find that changes in socioeconomic development are more important than climate change. This study improves our understanding of future changes to *Cryptosporidium* concentrations and could serve as the basis for better understanding future risk and disease burden.

#### P 145 Simulation techniques for high precision dose estimation in UV-LED water purification systems

Ashkan Babaie, Babak Adeli

Acuva Technologies Inc

Using virtual prototyping for the design of UV-LED reactors is significantly beneficial in reducing the product development cost. Without the proper simulation tools, the reactor design may need to go through multiple design revisions and prototyping stages without delivering the required UV dose or LRV at the end. Complete simulation of a reactor's performance requires precise modelling of reactor's hydrodynamics, optics and the microbial kinetics, each of which are equally important for an accurate reactor simulation.

Hydrodynamic simulation can be quite challenging if not all the parameters are considered or set properly. Meshing quality, flow models including turbulence, particle tracking setting can all

significantly affect the flow simulation and the dose performance, respectively. In fact, through examples, it will be discussed that traditional measures of simulation convergence as having the residuals below a certain level will not necessarily verify the accuracy of simulation. In addition, optical simulation will equally be important to achieve an accurate prediction of the reactor's performance. Precise simulation of the LED die, package, radiation pattern and wavelength, as well as modelling of optical surfaces will all influence the accuracy of the simulation tool.

In this study, a step-by-step approach for both optical and flow simulation within a UV-LED based water purifier will be discussed using commercial software packages such as Ansys and Zemax. Once reliable optical and hydrodynamic data are available, it will be explained how an integrated tool can be used to determine the overall reactor's UV dose delivery. Using the same approach, the simulation results will be compared with experimental bioassay test results, showing less than 10% variation. This level of accuracy can be achieved between experiments and simulation by paying extra attention to the detail of hydrodynamics, optics and kinetics simulation.

#### **P 146 Modelling physico-chemical and microbiological water quality - a geospatial analysis**

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Few interdisciplinary studies have been done on aquatic ecosystems using geospatial analysis to explain microbiological events. Changes in microbiological diversity remain largely undiscovered in most water bodies and with GIS, historic and future changes can be modelled and monitored. This study aimed to integrate land use, historic physico-chemical and microbiological water quality of a water body for improving future water management strategies. Objectives were to i) test water quality during two dry and wet seasons; ii) isolate and identify bacterial diversity from eDNA samples; iii) create contamination and microbial models. Physico-chemical parameters were determined with a multi-meter probe and Hach instrument. Microbiological analysis was done through membrane filtration and culturing methods and creating an antibiotic-resistance profile. eDNA extractions were done with a Nucleospin Soil (Macherey Nagel) extraction kit according to manufacturer's instructions. Historic and current water quality was visually represented using GIS and SWMM modelling software. Modelled results indicated the areas that have high sulphate ( $\pm 241$  mg/L) and nitrate ( $\pm 10$  mg/L) levels are prominent at the mining and urban areas and the pH levels ( $\pm 9.4$ ) are high in the dam area. A microbial model showed a correlation between the average *E. coli* counts and the distance travelled of the surface runoff of the watersheds. Antibiotic profiles indicated an increase in Multiple Antibiotic Resistances (MAR) at increased urban activities. The microbial diversity was determined using 16S rRNA gene (V3-V4) analysis of the eDNA with NGS on an Illumina MiSeq. Modelling the water quality demonstrated the value of combining geo-spatial and microbiological components for a holistic understanding of environmental health and risks.

#### **P 147 *Cryptosporidium* concentrations in rivers worldwide**

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*Cryptosporidium* is a leading cause of diarrhoea and infant mortality worldwide. A better understanding of the sources, fate and transport of *Cryptosporidium* via rivers is important for effective management of waterborne transmission, especially in the developing world. We present GloWPa-Crypto C1, the first global, spatially explicit model that computes *Cryptosporidium*

concentrations in rivers, implemented on a 0.5 x 0.5 grid and monthly time step [1]. To this end, we first modelled *Cryptosporidium* inputs to rivers from human faeces and animal manure. Next, we use modelled hydrology from a grid-based macroscale hydrological model (the Variable Infiltration Capacity model). Oocyst transport through the river network is modelled using a routing model, accounting for temperature- and solar radiation-dependent decay and sedimentation along the way. Monthly average oocyst concentrations are predicted to range from 10<sup>-6</sup> to 102 oocysts L<sup>-1</sup> in most places. Critical regions ('hotspots') with high concentrations mainly include densely populated areas in developing countries. Point sources (human faeces) appears to be a more dominant source of pollution than diffuse sources (mainly animal manure) in most world regions. Validation shows that GloWPa-Crypto medians are mostly within the range of observed concentrations. The model generally produces concentrations that are 1.5e2 log10 higher than the observations. This is likely predominantly due to the absence of recovery efficiency of the observations, which are therefore likely too low. Goodness of fit statistics are reasonable. Sensitivity analysis showed that the model is most sensitive to changes in input oocyst loads. GloWPa-Crypto C1 paves the way for many new opportunities at the global scale, including scenario analysis to investigate the impact of global change and management options on oocysts concentrations in rivers, and risk analysis to investigate human health risk.

1.Vermeulen, L.C., et al., *Cryptosporidium concentrations in rivers worldwide*. Water Res, 2019. 149: 202-214.

**P 148 Measuring Human Sewage Contamination in Recreational Surface Waters Using Human and Bacterial DNA Markers**

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Identifying the sources of fecal pollution in surface waters used for human recreation is necessary to reduce the potential for human contact with enteric pathogens that may pose serious risks to human health and for implementation of adequate pollution control and prevention strategies to mitigate pollution levels and protect water quality. Conventional fecal indicators such as Enterococcus and Escherichia coli are unreliable due to widely varying survival rates in the environment, failure to discriminate between sources, and inability to distinguish between fecal bacteria associated with recent contamination events and those adapted to secondary habitats. Innovative microbial source tracking (MST) techniques employing quantitative polymerase chain reaction (qPCR) have been developed to detect bacteria derived specifically from human feces, such as the human-associated Bacteroidales genetic markers, BacHum and HF183, which don't have ambiguous survival and sourcing issues and can provide good spatio-temporal resolution. The objective of this study was to identify the sources of human fecal pollution in an urban watershed located southeast of Boerne, Texas using general and human-associated fecal genetic markers, as well as human mitochondrial DNA (mtDNA) as a direct and robust human fecal marker. The results of the study confirmed the presence of human fecal contamination via the two human-associated Bacteroidales markers and mtDNA marker and suggested the major point sources of contamination were the effluent waters from two wastewater treatment plants. A decrease in the indicators occurrence inside of the preserve indicated the source of human fecal pollution to be coming from outside of the preserve. Rainfall data was unable to provide significant correlation with the occurrence of the markers, while flow data showed moderate to weak correlation with the human-associated markers and moderate correlation with the fecal indicator bacteria.

**P 149      Detection of human enteropathogens in recreational waters of Guanabara Bay, Rio de Janeiro, Brazil, by TaqMan Array Card (TAC)**

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A significant number of human enteropathogens are released into surface waters by point and non-point sewage discharges. Guanabara Bay is the second largest bay on the coast of Brazil. It has been suffering anthropogenic impacts for years, which led to poor environmental and sanitary conditions. This study aims to detect nineteen enteropathogens, including viruses, bacteria, and parasites, by an enteric TaqMan Array Card (TAC) in Guanabara Bay, Rio de Janeiro, Brazil. Twenty-six 10-L water samples were collected from 10 points of the Bay in 2015 and 2016. Enteropathogens were concentrated by an organic flocculation method and detected by TAC. Total coliforms, *Escherichia coli* (*E. coli*) and enterococci were quantified by Colilert and Enterolert as fecal indicator bacteria in all samples. Rotavirus, astrovirus, adenovirus, sapovirus, campylobacter, aeromonas, enterotoxigenic, enteroaggregative, Shiga toxin-producing, and enteropathogenic *escherichia coli*, shigella, bacteroides fragilis, plesiomonas, vibrio cholerae, blastocystis, entamoeba, encephalitozoon intestinalis, giardia, and *Cryptosporidium* were detected, being at least one of these enteropathogens detected in all samples. Adenovirus (15%), blatocystis (46%) and campylobacter (100%) were the most detected bacteria, parasite and virus, respectively. Fecal indicator bacteria results showed that all sample points were in accordance with the water quality standards, with exception of Marina da Gloria, and other two samples collected in 2016. Our data demonstrated the contamination of Guanabara Bay by different pathogens with importance to public health, which may represent a risk for those who are in contact with this faecally contaminated environment. It is also important to highlight that the presence of these enterophatogens in waters whose levels of fecal indicator bacteria were in accordance with regulatory standards. This research work is within the scope of the activities of FIOCRUZ as a collaborating centre of PAHO/WHO of Public and Environmental Health.

**P 150      The Development of a Water Quality Forecasting System for Recreational Coastal Bathing Waters in Ireland**

Daniel Hawtree, John O'Sullivan, Levent Görgü, Conor Muldoon, Gregory O'Hare, Bartholomew Masterson, Wim G. Meijer, Aurora Gitto, Malcolm Taylor, Elaine Mitchell

University College Dublin (School of Civil Engineering, School of Computer Science, School of Biomolecular & Biomedical Science); Agri-Food and Biosciences Institute

The revised European Bathing Water Directive (76/160/EEC 2006) requires the implementation of early warning systems for bathing waters which are subject to short-term pollution events. The EU SWIM project is developing coastal water quality prediction models and alert systems across the island of Ireland, which aims to provide short-range forecasts of bathing water quality. This system is being developed at nine beaches in the Republic of Ireland and Northern Ireland, which represent a range of baseline water quality and site conditions. This presentation provides an overview of the end-to-end prediction system, the development of the underlying models, and the challenges presented by such forecasting approaches.

Forecasts from this system are based on the relationship between faecal indicator bacteria and multiple environmental variables (e.g. precipitation, temperature, wind), which are used to generate predictions from ensemble decision tree models (i.e. random forest models). A unique aspect of this approach is the use of a historical back-cast climate data (Met Éireann's MÉRA dataset) as the foundation of model development, and the use of a related hourly climate forecast dataset (Met Éireann's Harmonie dataset) for predictions. This is able to generate hourly water quality predictions at any beach where

water quality data is available for model development.

The continuous stream of water quality predictions generated by these models are validated against data collected by routine sampling, as well as targeted supplementary water quality sampling, using a range of performance metrics (e.g. RMSE, AUC, sensitivity / specificity). Preliminary findings have shown promising model results at some sites, while models at other sites have yet to be successfully validated. The lack of validation at these sites may be attributed to several factors, including limited historical water quality data, the occurrence of non-meteorologically driven pollution events, and the high spatial and temporal variability of coastal water pollution.

## **P 151      Towards swimmable urban rivers: integrating risk management into riverside placemaking**

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A 'swimmable' river has become the desired outcome for urban river restoration projects, globally. However, a legacy of contaminated waterways in most cities presents a major barrier to the realisation of safe swimming sites. Faecal contamination is a primary source of health risk to recreational water users. Callan Park, in Sydney Australia is one of twelve proposed swimming sites within a vision to transform the Parramatta River into a recreational asset. Pathogens, such as viruses, bacteria and protozoa have several potential sources into the river. The most significant entry pathway is stormwater, which is contaminated with raw (untreated) municipal sewage during large rain events. Existing recreational water quality guidelines take into account such hazards, however these have traditionally been applied to coastal environments. This research aims to highlight limitations in the application of existing guidelines to polluted urban rivers, whilst investigating alternative assessment methodologies.

A microbial contaminant sampling program is underway at Callan Park, collecting 50 samples over nine months, conducted by Beachwatch NSW. This will inform a traditional microbial risk assessment. Limitations of this approach include the suitability of enterococci as an indicator for pathogenic viruses, and a rating system that does not account for dynamic changes in water quality. An alternative approach is to take lessons from the WHO Water Safety Plans, adopted globally as best practice for the provision of safe drinking water. This approach focuses on detailed assessment of catchment dynamics and environmental pathways for microbial contamination. Adopting this approach, this study is capturing the gradient of risk and the timescales in which it changes in riverine environments. Finally, management approaches to reduce exposure to microbial contaminants, in operation at urban river swimming sites internationally, will be identified. It is anticipated that the findings of this research will inform an improved approach to water quality assessment and assist waterway managers re-activate urban rivers for recreational use.

## **P 152      Microbial Dynamics and Human Health Risks at the Beach – Will Climate Change Matter?**

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Beaches provide recreational opportunities, relief from hot weather, and economic benefits to coastal communities. Visitors to the beach may be exposed to microbial contaminants and pathogens via water, sand, and aerosols. Water and sand coincide at the beach, providing an environment with unique advantages and challenges to pathogen introduction, growth and persistence, transport, and exchange among habitats. Advantages, such as refuge from predators in sand biofilms, and challenges,

such as the relatively dry environment, may be both exacerbated and complicated by seasonal variability and predicted long-term anthropogenic climate change. Human exposure to waterborne pathogens will likely be amplified in the face of predicted extreme rain events, warming of water, and sea level rise, combined with urbanization and the popularity of beach activities. Such changes may also alter microbial dynamics at beaches, potentially impacting assumptions and population relationships used in mechanistic water quality and *E. coli* concentration models as well as quantitative microbial risk assessment frameworks. With model refinement and parameter development designed to fill critical knowledge gaps, predictions of fecal indicator bacteria and pathogen fate and transport can help to characterize the risk of infectious disease from recreational water use. Here, we not only present a conceptual model that may serve as a first step toward inclusion of biofilm mechanics at beaches, but we also discuss potential approaches to incorporate sand-water interactions into hydrodynamic coastal models for enhanced beach health prediction.

While beach health and water quality have long been active areas of research, the sand and sand-water interface habitats at beaches remain relatively unexplored. Recent work has shown that sand can be a reservoir of microbial contaminants at beaches, signaling a potential paradigm shift in both research and management of recreational water and beaches to a more holistic, beachshed-based model, as further detailed in Weiskerger et al. (doi: 10.20944/preprints201901.0225.v1).

#### **P 153 Isolation of antibiotic resistant bacteria from tropical swimming pool sand filter backwash**

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The aim of the present work was to evaluate the presence of antibiotic resistant bacteria (ARB) in swimming pools from a tropical country. Sand filter backwash was collected from six swimming pools in Singapore and 2 milliliters of the concentrated backwash were plated in Nutrient Agar (NA), supplemented with antibiotics: carbenicillin (100 µg/mL), gentamycin (30 µg/mL), kanamycin (50 µg/mL), and tetracycline (15 µg/mL) to isolate ARB. After up to 120 hours at room temperature, colonies with distinct morphologies were isolated and sub-cultured until pure isolates were obtained.

The minimum inhibitory concentrations of seven antibiotics (covering 5 classes) were determined in nutrient broth with serial dilutions of carbenicillin (300 µg/ml), chloroquine (300 µg/ml), kanamycin (200 µg/ml), erythromycin (200 µg/ml), gentamicin (100 µg/ml), tetracycline (50 µg/ml), and ciprofloxacin (4 µg/ml). Optical density at 600 nm was measured to evaluate growth.

Genomic DNA from the isolates was extracted and amplified by PCR using universal 16S rRNA primers followed by Sanger sequencing. The sequences were searched against the NCBI-NR database, followed by phylogenetic tree construction. Taxonomic classification of the isolates was also performed using the RDP online portal's Naïve Bayesian Classifier.

A total of 41 isolates were obtained including 15 different species from 8 genera, with *Aeromonas* being the most abundant (10 isolates). Seventeen isolates were resistant to at least four antibiotics, with 11 of them resistant to the 7 antibiotics analyzed. Some of the isolates found, for example *Chryseobacterium* sp., *Elizabethkingia* sp., and *Stenotrophomonas* sp., had sequences closely related to known nosocomial pathogens.

Sand filtration is an effective method to remove microorganisms from swimming pool water. However, as the filter retains organic matter and solids, as well as microorganisms, it could become an ideal place for them to grow forming biofilms, where horizontal gene transfer is facilitated.

**P 154 Faecal pollution of surface waters in Dhaka city: potential risk of transmission of multidrug resistant *Salmonella* spp.**

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In underdeveloped countries, surface waters in urban areas are often polluted due to discharge of untreated sewage and stormwater containing faecal organisms. While epidemiological studies frequently implicate lakes and rivers as source of enteric infections, real health-risks remain unassessed due to inadequate environmental surveillance data. The present study was designed to monitor faecal contamination indicators in surface waters in Dhaka by analysis of water samples from the major river and an urban recreational lake. A total of 427 water samples collected from defined sites of Hatirjheel Lake and Buriganga River during winter and monsoon were analysed. Culture-based detection and estimation of total coliform (TC), fecal coliform (FC), and fecal streptococci (FS) showed maximum monthly average counts (cfu/mL) of 8.56E+3, 8.08E+2 and 6.56E+3 respectively and these counts fluctuated within one log-cycle throughout the study. FC counts often exceeded the EPA guideline value for Class III surface water (126 cfu/100mL) by a factor of >450. PCR-characterisation suggested that 77% of FS isolates were viridans streptococci implying humans being the major source (also reflected by average FS/FC ratio of 2.5), while 20% FS were  $\beta$ -hemolytic. PCR-confirmed *Salmonella* spp. were also isolated from 53% (n=159) samples. Within a subset of these isolates (n=30), 44% showed multiple antibiotic resistance phenotype and 67% could form biofilm on microtitre plates. The antibiotic resistance was horizontally transferable between different strains. Dhaka is a mega city with a population size of over 20 million, a large proportion of which do not have access to safe drinking water and adequate sanitation facilities. The widespread contamination of large water bodies due to poor management of faecal wastes puts the vulnerable population in urban Dhaka at high risk of gastrointestinal diseases. The evidence generated in this study highlights the necessity for prioritising the safeguard of environmental water reservoirs from faecal pollution.

**P 155 Sources, Transport Paths and Infection Risks during the use of Urban Surface Waters for Recreational Activities**

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Faecal contamination of urban surface water bodies poses a risk for public health. People can be exposed to faecal transmissible waterborne pathogens during recreational use of surface water bodies, which may lead to waterborne outbreaks. Waterborne Pathogens can enter surface waters from diffuse and point sources such as faecal deposits of animals, discharges from wastewater treatment plant effluents and combined sewer overflows. As an increase in storm events is predicted for the future, pathogen transport and infection risks are also likely to increase.

An interdisciplinary and integrative approach was developed for investigating the faecal contamination in urban surface water bodies and assessing the infection risks from exposure to waterborne pathogens during recreational activities at potential exposure sites in Vienna. The ultimate aims of this investigation are; to identify the potential dominant faecal pollution sources and pathways

affecting the microbial surface water quality in Vienna, to assess the infection risks for human associated with recreational use of surface water, and to develop a water-wise city planning tool for evaluating the effects of climate change, urbanization and the effectiveness of infection risk mitigation measures. A pollution source profiling (PSP) will be established to provide the total daily load of microbial cells and to reveal the potential dominant sources based on measured and reported source concentrations. The monitored data include faecal indicators, host-associated microbial source tracking markers, and reference pathogens. The PSP will serve as input to a new catchment-based water quality and infection risk model for the urban exposure sites following the QMRAcatch approach. Possible future scenarios will be investigated for deriving optimal strategies to achieve sustainable water safety. The approach follows the water-wise city concept of the IWA to support urban planning.

#### Acknowledgements

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#### **P 156      Quantification and prediction of *Vibrio cholerae* nonO1/nonO139 in bathing waters**

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*Vibrio cholerae* is a natural inhabitant of aquatic ecosystems with the potential of acting as a human pathogen. More than 200 serogroups have been described; so far, only serogroups O1 and O139 have been associated with epidemic cholera. However, also nonO1/nonO139 *Vibrio cholerae* strains are able to cause various other diseases, such as ear, wound, bloodstream as well as gastrointestinal infections or necrotizing fasciitis, with harmful impact on very young, elderly or weakened individuals.

In the past years, several cases of *Vibrio cholerae* nonO1/nonO139 infections have been documented in Austria with a local history, specifically associated with bathing activities. The observed increased number of local infections comes along with documented increases of infections in Europe and North America, most probably due to higher water temperatures triggered by global warming.

In an ongoing study the prevalence and abundance of *Vibrio cholerae* nonO1/nonO139 at selected bathing sites in Eastern Austria along spatiotemporal environmental gradients is being monitored. Solid quantitative data are obtained by culture based and culture independent (CARD-FISH/SPC and qPCR) methods, recently developed in our laboratory. Based on the linkage of these data with abundant environmental information, prediction models for the prevalence, spread and abundance of *Vibrio cholerae* nonO1/nonO139 in the bathing waters of the region shall be developed. This will - on the one hand - provide important information for national health authorities concerning the threat of *Vibrio cholerae* nonO1/nonO139 infections in bathing waters. On the other hand, the found correlations and models may also be transferred to other geographic regions.

**P 157 Reduction of Chlorine Inputs by Enhanced Degradation of Combined Chlorine via Electromagnetic Fields**

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Sanitation of recreational pool waters is vital to ensuring the health of those who utilize them, but often drastically increases the cost of upkeep, especially in resort style pools. Due to the risk of a health and publicity crisis that may arise from inadequate disinfection, pools are frequently over disinfected by maintaining high free chlorine residual in the form of hypochlorous ions ( $\text{ClO}^-$ ). However, this chlorine residual is reduced by environmental factors such as UV light or the addition of organic matter, urea, and various compounds that are introduced by those who utilize the pool. When free chlorine residual reacts with these nitrogen compounds chloramines are formed, resulting in lower overall disinfection power and free chlorine. As such, many companies aim to capitalize on this by proposing new technologies that claim to reduce chlorine consumption while maintaining acceptable levels of disinfection. One such technology to arise from this concept is the use of electromagnetic fields. Anecdotal evidence from one such company reports savings of up to \$50,000/yr in a 485,000gal pool in chlorine costs alone using a “drop-in” electromagnetic device that emits a constant magnetic field. We evaluated the influence of the device in replicated trials utilizing six 1700L swimming pools. Specifically, three pools with and three without the device were compared following the additions of chlorine and various organic or inorganic nitrogen compounds to the pool. Preliminary results of the study show that in the presence of the electromagnetic device at pH  $8\pm0.2$ , the chlorine demand of the pool system generated by the addition of  $\text{N}_2\text{H}_4\text{O}_3$  was reduced by 35% over the control within 30 minutes after the addition of ammonia. Study results indicate that this device degrades chloramine compounds resulting in a lower net chlorine input by reducing the magnitude of the breakpoint curve.

**P 158 Correlation of faecal indicator bacteria and potential pathogenic fungi on Dublin beaches and relation to public health**

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Sandy beaches are frequented and preferred for leisure activities, posing a risk to populations especially children, the elderly and individuals that are immuno-compromised. Sand and marine water are a reservoir of opportunistic and pathogenic microbial life as well as faecal indicator bacteria (FIB) that influence the bathing water quality status. The growth and the proliferation of microbes in beach sand and water are not restricted to bacteria but includes different groups of fungi such as potentially pathogenic and allergenic moulds, yeasts and dermatophytes. At present there is no clear guidance in relation to pathogenic fungi levels in these environments. Global warming and climate change also bring a regional de-characterization of the microbiota, due to the geographic expansion of endemic microbes.

This study aims to raise the profile of fungal communities for future regulation of microbial sand contamination and looks at correlating these with levels of FIB and their impact on human health.

Three sites of sandy beaches were selected in County Dublin, Ireland in urban and rural settings: Sandymount Strand, Dublin, The Brook Beach, Portrane and Balcarrick Beach, Donabate. The analysis was carried out for over one year to identify the possibility of seasonal variation. Sand samples were analyzed in triplicate to identify mycological agents (yeast, pathogenic fungi and dermatophytes). Water samples were processed to determine the presence of *Escherichia coli*, intestinal enterococci and fungi communities and physiochemical readings taken.

Our current data indicate that FIB levels in water vary by one order of magnitude. Seasonal variation can be seen in both the water and sand samples. Overall the composition of fungal groups does not differ from beach to beach. Further work will be carried out to determine which pathogenic fungi species are present on Dublin beaches.

## POSTER SESSION THURSDAY

### P 159      Occurrence of *Legionella pneumophila* in Drinking Water Distribution Systems

Mark W. LeChevallier

Dr. Water Consulting, LLC

Legionnaires' disease has increased by over 500 percent since 2000 and has become the most commonly identified cause of drinking water outbreaks in the U.S. Recently, IDEXX developed the simple-to-use Legiolert test to detect *L. pneumophila*. This study implemented the test at 10-12 utilities over a period of one year to assess the seasonal occurrence of *L. pneumophila* in drinking water distribution systems. A total of 1,348 samples were analyzed; 103 source water, 96 from the plant effluent, and 1,149 from the distribution system. Water quality parameters, including total coliform and *E. coli*, heterotrophic plate count bacteria, free or total chlorine residuals, temperature, pH, and total organic carbon levels were measured. *L. pneumophila* was detected in 5 raw water samples and not in any of the treated plant effluent samples. There was also no relationship between *L. pneumophila* and either total coliform bacteria or *E. coli*. Six of the 12 distribution systems examined had at least one positive *L. pneumophila* sample; 14 from free chlorinated systems and one from a chloraminated system. *L. pneumophila* occurred when water temperatures were >18°C. The highest concentrations occurred when chlorine residuals were less than 0.1 mg/L. *L. pneumophila* was no longer detected after the area was flushed and free chlorine residuals were maintained at levels >0.1 mg/L. These data suggest utilities should maintain a disinfectant residual of at least a 0.1 mg/L in all parts of the distribution system. The data showed that low concentrations of *L. pneumophila* may occur even when free chlorine residuals were more than 1.0 mg/L. The one chloraminated sample that was positive detected *L. pneumophila* at 4 MPN/100 mL with a residual of 3 mg/L total chlorine. A case study showed how a utility could focus capital resources on improvements that will better maintain disinfectant residuals throughout the system.

### P 160      Quantitative Microbial Risk Assessment to estimate the Public Health Risk from Exposure to drinking Water in Rural Area of Villapinzón, Colombia

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School of government Alberto Lleras Camargo - Los Andes University (Bogotá-Colombia)

Diarrhoeal disease caused by consumption of contaminated drinking water is an important cause of morbidity in rural areas. The failures, related with the treatment process, and operational monitoring are the major hazard source. The epidemiological evidence and the risk assessment are complementary tools to define water quality targets and define health based objectives. The aim of this research was to characterize the risk associated with the consumption of drinking water from rural communities of Villapinzón in 2015-2017.

A cross-sectional study with data base analysis was conducted, estimated the risk of diarrhea disease due to the consumption of contaminated drinking water from rural areas in five Villapinzón villages, based on the quantitative microbial risk assessment (QMRA) methodology associated with exposure to *Escherichia coli* natural contamination. Also, described the epidemiological evidence about diarrhoeal disease in the Municipality.

All sources showed *E. coli* contamination. The annual probability of infection was >10-4 for the five

villages with well and aqueduct water sources, Chigualá's population was safe when they drinking boiled water. 1318 records about diarrhoeal disease were registered in the municipality, majority in children above 5 years old and rural population.

The risk was not uniform between villages; the annual probability of infection was higher than the international reference level described by the Environmental Protection Agency. A water contamination pathway was found on household storage and home treatment.

This study provides the first approach to the estimation of the risk associated with water in rural area in Colombia. It also demonstrates the need to strengthen intersectional work, and implement improvements in rural water infrastructure to reduce the public health risk, and present technical information for the local government to plan prevention exercises that reduces the risk of exposure and the associated disease burden.

**P 161 Comparing microbial health risks associated with alternative approaches to managing sewer overflows using integrated modelling**

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Griffith University, Gold Coast, Australia, Water and Health, Salamander Bay, Australia, Water Technology, Brisbane, Queensland Urban Utilities, Brisbane, Australia

Wet weather sewer overflows pose potential health risks to exposed populations via residential manhole overflows, and subsequent downstream recreational waterway exposures. Pressures such as growing population, aging infrastructure and climate change challenge utilities with how to strategically manage and upgrade their infrastructure to provide the best outcome for the community in terms of costs for benefits. This study compared how selected public health risks change under different modelled management scenarios. Specifically, it considered how an expensive 'business as usual' sewerage upgrade would compare with an 'effects-based' approach with planned wet-weather sewage overflows. The Quantitative Microbial Risk Assessment (QMRA) framework was applied to assess the illness risk to downstream recreational users. Reference pathogen concentrations at three recreational sites were modelled for a range of wet-weather events (6, 12 and 24 month design events) under each management scenario, drawing on outputs of a hydrologic catchment model, a sewerage network model and a hydrodynamic model of the receiving waters. Additionally, we compared the impacts of these same management scenarios on the frequency, duration and magnitude of residential sewer overflows. Results predicted that the 'business as usual' and 'effects-based' management scenarios would be equally effective in significantly reducing residential sewer overflows (both reduced overflow volumes by >99%) while producing similar health risk profiles for downstream recreational waterway users. On the basis of these modelled health risk comparisons alone, planned wet weather overflows that are carefully managed and appropriately configured could be used to achieve significant capital infrastructure savings. It is important to note that these preliminary findings are based on a screening level health risk assessment. Further investigations are underway to consider the broader implications of moving away from the traditional management paradigm. This work shows that QMRA can play a significant role in understanding public health risks from sewer overflows and contribute to wider decision support risk assessments for sewer system management.

## **P 162 Antiretroviral residues in water: Implications for water safety testing?**

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Antiretrovirals (ARVs), used in the treatment of HIV infected people are entering the sewage treatment systems via human excretion. Ultimately effluent transports these substance into environmental waters systems. The aim of this study was to determine if the presence of ARVs affect the infective ability of coliphages and growth potential of bacteria used in quantitative microbial risk assessment (QMRA) methods. Exposure studies, in which *Escherichia coli* and coliphages interactions were investigated utilising three different classes of ARVs (separated by mechanism of inhibition) were performed. ARV concentrations ranging between 2 ng/mL and 80 ng/mL were previously detected in environmental and drinking waters and thus used in this study. These exposure studies included (i) establishing growth patterns of *E. coli* in the presence and absence of coliphages and ARVs; (ii) determining the reliability of the ISO 10705 method (international standard for enumerating coliphages in water); (iii) investigate the structural integrity of bacteriophages using transmission electron microscopy (TEM). Growth curve results indicated a decrease in bacterial growth/metabolic processes. TEM results indicated possible structural effects on bacteriophage appendages. These two observations could explain the decrease in phage levels established by ISO 10705, as well as the uneven formations of bacterial mats. The ISO 10705 method is the most cost effective and widely implemented method for QMRA concerning viral safety of water. Information gathered by this method is used in surveillance and monitoring studies. Such impacted information of phage levels could have further reaching impacts on water quality management decisions and practices. Ultimately, this could have an impact on how agencies tasked with water safety management and policies in regards to United Nations Sustainable Development Goal 6 (Clean water and sanitation for all) perceive the safety of their water catchments.

## **P 163 Effect of climatic and demographic changes and the upgrading of wastewater infrastructure on the bacterial faecal pollution of a large river and on the associated health risks**

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Water safety planning requires a systemic approach, including how risks may change in the future and what management options might be taken. The model QMRAcatch allows answering such questions by integrating a catchment-based microbial fate and transport model with quantitative microbial risk assessment.

In this study we investigated how future climatic and demographic changes will affect the bacterial faecal pollution burden of the Danube River in Vienna, as indicated by the concentration of *E. coli* in river water. We also assessed what improvements may be expected by upgrading the wastewater infrastructure in the catchment upstream. Finally, we evaluated how these changes would affect the health risk associated with bathing and the treatment requirements for producing safe drinking water from river water, with respect to infections from the bacterial reference pathogen *Campylobacter*.

Our results show that the projected population increase together with the estimated changes in the hydrological regime of the river and in water temperature will have a negligible effect on the bacterial faecal pollution of the river in our study area. The upgrading of the wastewater infrastructure, if carried out at all faecal pollution sources, would however bring substantial improvements in microbial water quality, reflected by an improvement in the bathing water classification and by a lowering of the risk of infection from bathing related to the reference pathogen *Campylobacter*. The treatment requirements for safe drinking water show a similar pattern. This study highlights the importance of catchment protection in the sustainable management of public health risks associated with bacterial waterborne diseases.

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**P 165 Risk-based water quality threshold for coliphage in surface waters of different temperatures**

Alexandria Boehm

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This study investigated the risk of gastrointestinal illness associated with swimming in surface waters with aged sewage contamination using quantitative microbial risk assessment (QMRA). The QMRA used F+ and somatic coliphage as indices for the amount of sewage present and thereby provided insight into how risk relates to coliphage concentrations in surface water. We compiled data on temperature-dependent pathogen and coliphage decay rate constants in surface waters using a systematic review and subsequent meta-analysis. We conducted the systematic review following PRISMA guidelines. The meta-analysis explored how water temperature, sunlight, water salinity, and pathogen measurement method are associated with decay rate constants. The compiled decay rate constants allowed for us to account for differential decline in pathogen and coliphage concentrations as they age in the surface water in the QMRA model. Because exposure to norovirus contributed the majority of risk, and coliphage decay rate constants are greater than norovirus decay rate constants, the risk associated with exposure to a fixed coliphage concentration increases with the age of contamination. Swimmer exposure to sewage after it has aged approximately 3 days results in median risks less than 30/1000 which is the USEPA benchmark risk. A risk-based water quality threshold for coliphage in surface waters that takes into account uncertainty in contamination age, and water temperature is derived. This framework can be applied to assessing risk associated with indicators from other single microbial pollutant sources including treated wastewater and agricultural runoff. The framework can also be applied to a pollution scenario where there are multiple pathogen sources (for example, a mixture of gull feces and raw sewage).

**P 166 Transfer of water and *E. coli* to the skin after liquid-contact activities**

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Infectious diseases are one of the leading causes of death across the world and human interactions with contaminated liquids (e.g., liquids containing bacteria) increase the risk of infection. After skin-liquid contact, bacteria present in contaminated water are transferred to the skin via adsorption onto the skin or due to residual liquid retention on the skin. Available estimations of the volume of residual liquid retained are uncertain and incomplete. Additionally, only data on virus adsorption is available to estimate pathogen transfer during liquid-contact activities, having to rely solely on this for risk

assessments that involve bacteria. The objectives of this study were to quantify the volume of liquid retained on the skin following various activities and to quantify the number of bacteria transferred to the skin after liquid-to-skin contact. To this aim, we performed two sets of human subject studies. In the first study, 40 adult volunteers performed 3 different activities involving hand-to-liquid contacts and the volume of liquid transferred was quantified. In the second study, *E. coli* transfer studies were performed with 7 adult volunteers. Volunteers retained on average 0.0039, 0.0046, and 0.0076 cm<sup>3</sup>/cm<sup>2</sup> liquid on the skin after wet-cloth contact, wet-surface contact, and hand immersion respectively. Factors such as the activity performed, the surface material touched, the way of contacting the material influenced the volume of water remaining on the skin. Additionally, the adsorption of bacteria onto the skin was estimable using linear regression model,  $n = C^{1.2+} 10^{-4.6}$ , where n is the number of bacteria per surface area, C is the concentration of *E. coli* in the liquid, with the coefficients derived empirically. Overall, this study presents estimates useful to develop microbial risk assessments for recreational and occupational water exposure.

**P 167 Improvement of probabilistic QMRA by quantitative integration of external information using Bayesian hierarchical modelling**

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Probabilistic QMRA studies define model inputs as random variables and use Monte-Carlo simulation to generate distributions of potential risk outcomes. However, in many QMRA studies, local data is limited or completely absent. In such situations, distribution parameters for these random variables cannot be estimated precisely. Appropriate parameter estimates, however, are crucial for the robustness of simulation results and thus affect risk-based decision-making. In QMRA studies, deriving assumptions about point estimates for these parameters from the literature is a widely accepted approach when local data is completely absent. However, the extent to which external information should be allowed to influence local estimates against the background of nonexistent, scarce, and moderate local data remains unexplored. This question can be addressed by employing Bayesian hierarchical modelling; however, QMRA studies rarely make use of this technique.

This study focuses on the effects and potential benefits of Bayesian hierarchical modelling on risk and performance target calculations at three demo-sites (restricted irrigation, potable reuse, recreational waters). We also quantify the extent to which external data affects local risk estimations in each case. As reference pathogens and example data, we use locally collected norovirus II data with varying sample sizes ( $n = 4$ ,  $n = 7$ ,  $n = 27$ ), and complement local information with external information from 44 WWTPs published in (Eftim et al. 2017) ( $n = 305$ ). Results show that hierarchical modelling improves estimates by reducing parameter uncertainty when data are scarce, and may affect risk and performance target calculations by orders of magnitude. Furthermore, it allows making generalizable inferences about new WWTPs, while providing the necessary flexibility to adjust for different levels of information provided by the locally available data. Applying this flexible technique more widely may contribute to improving methods and standards in future QMRA studies.

**P 168 Pathogenicity of *Salmonella* and *Campylobacter* in recreational waters: new insight from the Yarra River**

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Health risks to recreational river-users can be estimated using Quantitative Microbial Risk Assessments (QMRAs). Some QMRAs make assumptions about the proportion of organisms detected in the water that are capable of infecting humans. Unfortunately, there is almost no data to reinforce these %infective assumptions, resulting in worst case scenarios being adopted (100% infective). Our work builds an evidence base regarding the infective proportions of *Campylobacter* and *Salmonella* detected in urban river systems. *Salmonella* and *Campylobacter* isolates were recovered from water and sediments of the Yarra River and its estuary during winter and summer. These isolates were used for quantification of attachment and invasion potential using differentiated Caco-2 monolayers. *Campylobacter* was also tested for cytotoxicity on HeLa-cells. Results suggest that all estuarine *Salmonella* (n=62) could attach and invade Caco-2 monolayer; 95% confidence interval (CI) of attachment-efficiency was 8.6%-10.5% and invasion-efficiency was 0.14%-0.22% (compared to inoculum concentrations). Relative to the 5th percentile value for the *Salmonella* Typhimurium ATCC14028 control, 32% and 50% isolates had lower attachment and invasion potentials respectively. For *Campylobacter* (n=189), 95% CI for attachment efficiency varied from 0.09% to 0.1% (96.3% had lower efficiency than the 5th percentile value for *Campylobacter jejuni* 11168). None of these strains invaded Caco-2 monolayers. Regarding HeLa-cytotoxicity, 95% CI titres for the estuarine *Campylobacter* (n=95) was 61-85 and none showed titre higher than the 5th percentile value observed for *C. jejuni* 11168. While environmental factors such as sunlight, temperature, and salinity might have partially influenced Caco-2 attachment and invasion capacities for *Salmonella* and *Campylobacter*, the observed heterogeneity was likely inherent and possibly associated with diverse sources of these organisms to the estuary. Considering the multiple virulence factors and different methods used to determine pathogenic potential, this study highlights the potential shortcomings of assuming all environmental strains are 100% virulent as part QMRAs.

**P 169 Evaluation of Risk associated with *E. coli* (pathogenic) in Stored Water Using QMRA : A Real-time Surveillance of an Urban Slum Pocket, Mumbai City, India**

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The study aims to quantify the health risk associated with *Escherichia coli* (pathogenic) in stored water used for drinking and food preparation using Quantitative Microbial Risk Assessment (QMRA) coupled with assessment of behaviour of mothers of <5 year old children towards Water, Sanitation and Hygiene (WASH) practices in an urban slum. The risk of infection from *Escherichia coli* (pathogenic) ranged from 10-2 to 10-4 per year and the reported enteric illness was 44% during 18 months of study period. The calculated health burden due to *Escherichia coli* (pathogenic) ranged from 10-3-10-5 DALY/person/year, higher than the WHO reference level of 10-6. Through a questionnaire and water analysis, we identified key risk factors for enteric disease in children <5 years of age. In the household, presence of *Escherichia coli* (pathogenic) in water was significantly associated with material used for storage of water for food preparation ( $p=0.049$ ) and its transfer ( $p=0.05$ ). Presence of diarrhoea was significantly associated with material used for storage of water for food preparation ( $p=0.033$ ) and visual appearance ( $p=0.002$ ). Age of mother ( $p=0.030$ ) and knowledge of mothers on diarrhoea ( $p=0.025$ ) were significantly associated with hand washing practices. Significant association was observed between education of mother with treatment of water ( $p=0.002$ ) and use of toilet facility ( $p=0.007$ ) for children.

An awareness program on WASH practices was conducted for mothers. 61% of study households were able to attend the program. Remaining households were briefed on a one-to-one basis in their house. An interventional study to improve point of use water quality and reduce diarrheal infection among <5 years old children started in Apr 2019 will be completed by Dec 2019. The households were divided into 3 groups viz. Test 1 (provision of water purification device), Test 2 (provision of earthen pot with lid and tap) and control group.

**P 170 Real time *E. coli* activity monitoring in reclaimed water as a tool to manage microbiological risks**

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Microbiological risks need to be assessed and controlled in order to be able to use reclaimed water safely. Sanitation Safety Plans (SSP) become important in this framework and new emerging technologies, such as microbiological online analysers, are appearing to improve them.

With the aim of improving the microbiological risk management during the reclaimed water production, an *E. coli* online analyser (BACTcontrol, MicroLAN), was evaluated. BACTcontrol analyses *E. coli* living cells enzymatic activity via fluorescence detection, every 2 hours. BACTcontrol has a self-cleaning system to avoid regrowth and cross contamination, and a sample concentrating system to improve the sensitivity. The evaluation had two parts:

-Under controlled conditions (offline analysis): BACTcontrol analysed dechlorinated tap water and reclaimed water spiked with waste water offline samples for 3 months. Colilert® method and physicochemical parameters were used to evaluate the data obtained.

-Field evaluation at Waste Water Treatment Plant (WWTP) (online evaluation): BACTcontrol was installed in Gavà-Viladecans WWTP after the tertiary treatment (Membrane Biological Reactor, with a production of 15.000 m<sup>3</sup>/day) for 6 months. Colilert® method, online physicochemical parameters and events in the operation of the plant were used to evaluate the data obtained.

After this exhaustive evaluation about how microbiological online analysers can improve the risk management of the reclaimed water production, the main results to present are the detection limit of BACTcontrol, *E. coli* activity values' ranges in reclaimed water as well as showing the potential that this type of analysers have to help to manage the microbiological risks by reporting real time monitoring of *E. coli* activity, which can benefit SSP designing.

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**P 171 Identifying challenges in estimating risks of infection due to antibiotic-resistant pathogens: hazard and dose-response relationship trends**

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Unplanned antibiotics usage increased the global spread of antibiotics resistance genes and their human interaction upsurges the morbidity. Literature shows majority of the disease causing bacteria are multiple drugs resistant (MDRB). Thus, it becomes important to estimate infection chances due to these pathogens. This study aimed to identify challenges in estimating infection risk as exposures of antibiotic-resistant pathogens and understand the pathogenicity (infectivity potential and approach) of MDRB/non-MDRB to humans. Hazard identification step analysis shows samples from raw and finished drinking water displayed different values of MDR/non-MDRB.

Study indicates out of  $1.19 \times 10^6$  CFU/mL screened for raw water, 58% were found to be MDR and 89% of bacteria out of 68 CFU/mL in drinking water were MDR. Similarly, another research revealed a population of 100 CFU/100 mL of drinking water contains 40-70 MDRB. Furthermore, empirical data of dose-response relationship is available for non-MDRB, but not for MDRB. It is important to know if the already existing dose-response relationship of non-MDRB can be used for MDRB until we get experimental data for developing a required dose-response curve. The uncertainty remains on information about ability of human body to (i) reject pathogens, (ii) show early signatures of infection after MDR encounter? Is the pathogenicity will be the same for both or not? If the infectivity potential is higher for MDR pathogen, there is a need to revise the existing dose-response curve (like:  $P_{infectivity}=1-e^{-kd}$ ) and recalculate it by using a proposed correction factor ( $f \sim$  infection rates as per the resistant data regarding the MDRB exposure). The revised dose-response curve could be written as:  $P_{infectivity}(ARB)=P_{infectivity}*(f)=1-e^{-k1d}$ . And more databases is required on (i) fractions of MDR/non-MDRB in different environmental samples, (ii) extent of antibiotics resistance within human for estimating  $f$ -value; estimating risks of infection due to antibiotic-resistant pathogens which has not been valued so far.

**P 172 Quantitative risk assessment of norovirus and adenovirus for the use of reclaimed water to irrigate lettuce in Catalonia**

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Wastewater is an important resource in water-scarce regions of the world, and its use in agriculture requires the guarantee of acceptable public health risks. The use of fecal indicator bacteria to evaluate safety does not represent viruses, the main potential health hazards. Viral pathogens could complement the use of fecal indicator bacteria in the evaluation of water quality. In this study, we characterized the concentration and removal of human adenovirus (HAdV) and norovirus genogroup II (NoVGII) in two wastewater treatment plants (WWTPs) that use different tertiary treatments (constructed wetland vs conventional UV, chlorination and Actiflo® treatments) for a year in Catalonia. The main objective of this study was to develop a QMRA for viral gastroenteritis caused by NoVGII and HAdV, associated with the ingestion of lettuce irrigated with tertiary effluents from these WWTPs. The results show that the disease burden of NoVGII and HAdV for the consumption of lettuce irrigated with tertiary effluent from either WWTP was higher than the WHO recommendation. The WWTP with constructed wetland showed a higher viral reduction on average (3.9 and 2.8 logs for NoVGII and HAdV, respectively) than conventional treatment (1.9 and 2.5 logs) but a higher variability than the conventional WWTP. Sensitivity analysis demonstrated that the input parameters used to estimate the viral reduction by treatment and viral concentrations accounted for much of the model output variability. The estimated reductions required to reach the WHO recommended levels in tertiary effluent are influenced by the characteristics of the treatments developed in the WWTPs, and additional average reductions are necessary (in WWTP with a constructed wetland: 6.7 and 5.1 logs for NoVGII and HAdV, respectively; and in the more conventional treatment: 7 and 5.6 logs). The results suggest that the analyzed reclaimed water would require additional treatments to achieve acceptable risk in the irrigation of vegetables with reclaimed water.

**P 173      Quantitative microbial risk assessment of enteric viruses in recreational waters in Vitória, Brazil**

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Brazil is covered by mangrove all along its coastline and a significant part of it is located nearby urban areas. The lack of wastewater treatment as well as the several non-point sources pollution may contaminate mangrove waters and pose high microbial risk to local residents through bathing activities. Recent studies have observed high levels of *Escherichia coli* as well as pathogenic microorganisms in Vitória Bay, which is enclosed by an extent mangrove area. Therefore, the local population may be exposed to a high-risk level of developing enteric diseases through recreational activities. The aim of this study was to assess the microbial risk posed to bathers by adenovirus, norovirus and rotavirus and estimate the risk reduction achieved by the hypothetical implementation of wastewater treatment technologies. A water monitoring programme was implemented for a one-year period and samples ( $n=36$ ) were monthly collected from three distinct points located in Vitória Bay. Water samples were analysed for *E. coli* and *Enterococcus spp.* using culture-based method, and for adenovirus, norovirus and rotavirus using molecular assays by real-time PCR. Data collected during the monitoring programme were used to feed quantitative microbial risk assessment (QMRA) models. High frequency of pathogenic viruses was observed in water samples (69.4%). The highest mean concentration of pathogens was observed for rotavirus (2.44E03 genome copies.100 mL<sup>-1</sup>), whereas norovirus presented the lowest (1.68E01 genome copies.100 mL<sup>-1</sup>). The QMRA revealed that bathers were exposed to a relatively high risk of infection ( $> 1.00E-4$ ) posed by all viruses studied. The implementation of a wastewater treatment plant, including a disinfection step, was shown to be the most appropriate intervention to reduce the risk of infection posed by all target pathogens. The findings of this research are expected to support government interventions in sanitation to protect waters used for recreational activities.

**P 174      Infectious Enterovirus in the Danube River**

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Bank filtrate of large rivers like the Danube very often serves as drinking water resource. Comprehensive knowledge of the potential health risks originating from the respective river water is crucial for water safety planning. In this regard quantitative information about the occurrence of human infectious microorganisms and viruses in particular play a vital role. Enteroviruses not only are major, water-transmittable human pathogens, but also very suitable reference ones. Since an appropriate quantitative cell culture system is available to discriminate between intact, infectious virus particles and degraded, non-infectious ones, enteroviruses give most reliable data for health risk assessment.

In this study surface water samples from four different Danube River sites along and upstream Vienna were investigated monthly for a period of four and six years, respectively ( $n=216$ ). BGM (Buffalo Green Monkey) cell cultures were used to detect enteroviruses and quantitation was done by an MPN (most probable number) method. Data obtained show the presence of infectious enteroviruses in 35%-48% of the water samples (mean 44%). The majority of positive records occurred in the winter season

(October-March), i.e. 64%-90% of the positive results (mean 76%). Moreover remarkably higher viral concentrations were observed in winter than in summer season (Mean winter 6.26; Mean summer 0.80; Median winter 6.26; Median summer 0.00 MPN-CU/10l).

For the first time thorough information about the prevalence and abundance of infectious enteroviruses in Danube River waters throughout the year is available. These data are essential for risk assessment and guiding sustainable measures for water safety planning.

#### Acknowledgments

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#### **P 175 Water Safety Plan implementation in a Water Supply Company of Campinas City, São Paulo State, Brazil.**

Diego de Oliveira Pinto, Adriana A. R. V. Isenburg, Romeu Cantusio Neto

SANASA - Society for Water Supply and Sanitation S/A

The Society for Water Supply and Sanitation (SANASA) company is responsible for water supply and wastewater treatment in Campinas, São Paulo State, Brazil. This city belongs to the Piracicaba, Capivari and Jundiaí (PCJ) rivers basin, a highly industrialized and populous region with 5.418.961 inhabitants<sup>1</sup>. SANASA is ISO 9001/2015 certified, and internationally recognized for its excellence in sanitation area, complying with the relevant Brazilian legislation. In 2018, SANASA was the first among the municipal sanitation companies in Brazil, according to the Environmental Sanitation Journal<sup>2</sup>, a leading publication in the sanitation sector. In the last years, SANASA has focused on drinking water safety setting up the Water Safety Plan (WSP) for Campinas in March 2012. In 2019, it became a coordinating board which has quality control plans linked to the contingency plans that enable the identification, classification and risk management based on the HACCP system, as well as real-time monitoring of the multiple barriers performance for each control point, critical control point or attention point previously determined according to the decision tree<sup>3</sup>. It works by direct investigation on each stage of the water supply system, from the catchment to the point of consumption, through the validation of the control measures and verification of the final product. Concomitantly, SANASA is structuring an international center for water research and innovation (Brazilian Water Research Center - BWRC). That will bring relevant contributions to the study of Quantitative Microbiological and Chemical Risk Assessment, as well as studies focused on PCJ basin, water quality and availability for drought periods. The BWRC will be done in partnership with State University of Campinas (UNICAMP) and the Foundation for Research Support of São Paulo State (FAPESP).

#### **P 176 Occurrence of antimicrobial resistant bacteria and resistance genes in surface water, groundwater and drinking water in Germany**

Claudia Stange and Andreas Tiehm

DVGW-Technologiezentrum Wasser

Due to the widespread use of antibiotics, resistances have spread in the environment. Resistant bacteria are also increasingly found in the population, unrelated to hospitalization, suggesting the transfer of resistance genes from the environment into humans.

In this study, the presence of antimicrobial resistant bacteria (ARB) and antimicrobial resistance genes (ARGs) in different types of raw waters used for drinking water production was examined. Total DNA extracts were analyzed for eight ARGs via qPCR. Culture methods based on selective CHROM agar plates containing antibiotics were used to prove the presence of vancomycin-resistant enterococci (VRE), extended-spectrum beta-lactamase (ESBL) producing bacteria and methicillin-resistant

*Staphylococcus aureus* (MRSA).

Analysis of samples using culture based methods demonstrated the presence of ESBL producing *E. coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Acinetobacter*, and *Pseudomonas* as well as VRE in surface waters. ESBL producing *E. coli* and VRE could also be detected in groundwater - in exceptional cases. Drinking water was free of the analyzed ARB. qPCR analysis of raw waters demonstrated the wide distribution of some ARGs (*sul1*, *blaTEM*, *ermB*, and *blaCMY-2*) in surface water. Other ARGs were not detected or only in low frequency (*blaCTX-M-32*, *mecA* and *mcr-1*). In drinking water only the sulfonamide resistance gene *sul1* was detected.

The results demonstrate the distribution of ARB and ARGs via the water pathway. According to the UN sustainable development goals, mitigation of resistances spreading and improvement of raw water quality are encouraged.

The study was funded by the German Federal Ministry of Education and Research (HyReKA, 02WRS1377G).

**P 177 Removal of antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARGs) during drinking water treatment**

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DVGW-Technologiezentrum Wasser, DVGW-Technologiezentrum Wasser, CSIRO Oceans and Atmosphere, CSIRO Land and Water

In this study, the removal of ARB and ARGs by different drinking water treatment processes was examined. Chlorination, ozonation, and UV treatment were investigated on a laboratory scale using *Escherichia coli* and *Enterococcus faecium* carrying ARGs. Bacterial inactivation was determined by plate count methods, and ARGs were quantified using qPCR. For ozone, similar elimination rates were observed in terms of cultivable bacteria and ARGs. For chlorine and UV, inactivation of bacteria was observed at lower doses as compared to the doses required for a decrease in ARG copy numbers. Whereas UV and chlorine treatments were effective in ARB inactivation, ARGs were not completely removed. However, gene copy numbers after treatment were lower if long amplicon qPCR was applied.

In full scale water utilities, a reduction of the ARGs was observed by the drinking water treatment process. Not only the reactive techniques (i.e. UV, ozone, chlorine disinfection) led to a decrease, but also the non-reactive treatment steps (such as flocculation and filtration) contributed to the decline of ARGs. However, in few samples ARGs were increased after biological activated carbon filters, most probably due to biofilm flocs mobilization.

In conclusion, the study demonstrates reduction of ARB and ARGs by drinking water treatment with varying efficiencies. More field studies into antibiotic resistances and application of harmonized methods are encouraged to better understand loads and removal rates, depending on treatment process and operation conditions.

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**P 178 Pilot-scale Comparisons of Different Advanced Oxidation Processes for Removing Viable Antibiotic Resistance Bacteria from Domestic Wastewater**

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Antibiotic resistance bacteria (ARB) and genes (ARGs) can spread through the environment, often associated with releases of domestic and other wastewater. Although conventional wastewater treatment technologies are efficient at removing pathogens including most ARB and ARGs, additional treatment steps may be needed when conventional treatment is not adequate to satisfy local water quality needs, which is most apparent in water scarce areas and/or where reuse is needed. In this context, advanced oxidation processes (AOPs) have shown potential in removing recalcitrant compounds from wastewater, but different options have not been systematically evaluated for removing ARB and ARGs in pilot-scale systems. Here we evaluated and compared three AOPs (i.e., ozonation, H<sub>2</sub>O<sub>2</sub> coupled with UV, and their combination) in their relative ability to reduce ARGs diversity and abundances from treated secondary effluent. Specifically, over 300 ARGs were monitored using high-throughput quantitative polymerase chain reaction and ARG mass balances were performed on viable and non-viable cells (using propidium monoazide methods to quantify ARGs in viable cells). Ozonation at a conventional dose of 3 g/m<sup>3</sup> displayed the highest removal in both numbers of unique ARGs and abundances, achieving ARGs removal of 2 orders of magnitude. In contrast, the combined H<sub>2</sub>O<sub>2</sub>/UV/O<sub>3</sub> treatment did not have any significant additional effect on the reduction of ARGs diversity and abundances. Ozonation also achieved highest reductions in the viable/non-viable ratio (0.44), confirming that this technology had greater impact on viable ARB than non-viable ARB treated effluents. This is very promising because any technology that can enhance the die-off of viable cells carrying ARGs will almost certainly reduce ARG gene exchange in released wastewaters. More work is needed on ARB viability in wastewater treatment technologies, but here we show that ozonation reduces the viable ARB fraction in pilot wastewater systems, suggesting it has value for enhanced wastewater treatment, especially for reuse.

**P 180 Potential public health hazards in green stormwater infrastructure: Microfluidic quantification of pathogens and antimicrobial resistance genes**

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Pathogens and antibiotic resistance genes (ARGs) have been quantified in a variety of stormwater sources. Additionally, stormwater runoff can contain heavy metals and potential biological stressors that can further contribute to environmental reservoirs or “hot spots” of antibiotic resistance. However, to date, no studies have quantified ARGs in low impact development or “green” stormwater infrastructure, and information regarding pathogen fate and transport in these installations is scarce. Green infrastructure (GI) primarily makes use of soil and other engineered materials to increase sustainability compared to traditional “gray” stormwater conveyance infrastructure by allowing for infiltration similar to natural environments. GI installations can include rain gardens, planter boxes, bioswales, permeable pavements, and green roofs, and are often combined with multi-use public spaces. While GI provides more community and ecosystem benefits compared to gray infrastructure, potential microbial hazards may be present in stormwater-impacted soils that warrant further investigation in order to maximize the potential benefits and reduce potential risks of sustainable stormwater infrastructure. To address these gaps, 46 antibiotic resistance genes, 8 pathogens (*Campylobacter* spp., *Pseudomonas aeruginosa*, *Legionella longbeachae*, *Cryptococcus gaati*,

*Cryptococcus neoformans*, *Acanthamoeba* spp., ringworm, and *Mycobacterium intracellulare*), and 2 fecal indicator bacteria (*E. coli*, *Enterococcus* spp.) were quantified in 93 topsoil samples from 14 green infrastructure installations from urban Philadelphia using microfluidic qPCR. Samples were positive for *E. coli* (88%), *Enterococcus* spp. (87%), *M. intracellulare* (100%), *P. aeruginosa* (6.5%), and *Campylobacter* spp. (100%). Additionally, 43 of 46 antibiotic resistance genes were positive in at least one sample. GI installations therefore contained potential microbial hazards, and although exposure is not well understood may warrant further exploration, especially compared to non-stormwater impacted soils. Gene concentrations are put into the context of a systematic literature review of pathogens and antibiotic resistance genes in stormwater runoff. Risk assessment methods for quantifying antibiotic resistance risks in stormwater infrastructure are proposed.

**P 186 Small Urban Streams and Urban Bathing Waters are Impacted by Faecal and Antibiotic Resistance Gene Pollution**

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Antibiotic resistance is the greatest threat to our healthcare systems. One Health recognises that the environment is shared by humans and animals and that it is a route by which bacteria harbouring antibiotic resistance genes can establish themselves in humans. Small urban streams that do not receive treated waste water and urban bathing waters have received little attention but may be an important source of antibiotic resistance genes.

We aimed to assess levels and sources of faecal pollution in small urban streams that do not receive treated waste water and their impact on bathing waters. We also wanted to determine antibiotic resistance gene levels in these streams and bathing waters, and if these levels correlated with levels of human and animal faecal markers.

For one year, monthly water samples were taken from Dublin city's three designated bathing waters and two urban streams that discharge onto them. Dublin's largest river that receives treated waste water was also sampled. The levels of faecal indicator organisms (*E. coli* and intestinal enterococci) were ascertained and the source of faecal pollution (human, dog, gull) quantified by microbial source tracking. Also, the levels of genes conferring resistance to clinically important  $\beta$ -lactam, sulfonamide, fluoroquinolone and tetracycline antibiotics were quantified by qPCR.

Our results demonstrate that these urban streams are impacted by human faecal pollution and antibiotic resistance gene levels correlated with faecal indicator levels in the streams. Additionally, no significant differences were observed between the small urban streams and the waste water impacted river in terms of faecal indicator and relative antibiotic resistance gene levels. Furthermore, the bathing zones examined frequently failed to meet excellent water quality and contained high levels of antibiotic resistance genes. These urban streams and bathing waters thus pose a public health risk as they may transmit enteric pathogens and antibiotic resistance determinants.

## **P 187      Antibiotic-resistant bacteria in patient wet rooms of a maximum care oncology ward**

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The importance and health relevance of the increase of antibiotic-resistant bacteria (ARB) was highlighted by the G7 summit in 2015 and the 2017 G20 summit, as well as by experts of a WHO workshop during the WaterMicro conference 2015 in Lisbon/Portugal.

The occurrence and spread of antibiotics and ARB in the aquatic environment can be caused by human and animal excretions. Among different emissions pathways, wastewater is suspected to be one of the most important point sources of ARB, and hospitals are suspected to be possible hotspots.

A sub-study of the multidisciplinary collaborative research project "HyReKA" (BMBF, funding code 02WRS1377) dealt with the characterization of the clinical-human pathway with respect to antibiotic-resistant pathogens. Wastewater of a maximum care hospital was examined.

To characterize the situation, shower drains, toilets and sink drains from patient wet rooms of an oncology ward were examined. Following the initial screening of the whole ward, three patient wet rooms were examined every six weeks over twelve month to investigate a possible seasonal course. Each sample included not only the waste water but also biofilm material from the walls of the drains. The samples were tested for about 40 antibiotics and different ARB. The cultured method used in medical microbiology for the detection of Extended Spectrum Beta-Lactamase producing gram negative bacteria, Methicillin-resistant *Staphylococcus aureus*, and Vancomycin-resistant enterococci in human material had to be adapted and optimized for analysis of environmental samples. The characterization of the isolates and their resistance properties were carried out using molecular biological methods.

High concentrations of antibiotics and their residues were detected (Voigt et al., 2018; DOI: 10.1016/j.ijheh.2018.12.013). Culturing results show ARB and multi-resistant isolates in varying abundance and frequency. Some of the strains could only be detected temporally and spatially limited; others were almost constant present throughout the whole study period.

## **P 188      Low-energy wastewater treatment for reducing resistant bacteria**

Andrew M. Zealand, David W. Graham

Newcastle University

A lack of adequate sanitation and the resulting contaminated waters can lead to the spread of infectious diseases and antimicrobial resistance (AMR) in the environment and population. However, wastewater treatment in developing countries is often unavailable or underfunded. Currently, small, per-urban areas in Malaysia in need of wastewater treatments often employ a mixture of extended aeration, Imhoff tanks, and activated sludge; none of which can regularly achieve the outcomes previously mentioned.

A down-flow hanging sponge (DHS) system was built in Johor Bahru, Malaysia, to compare its removal of the traditional wastewater parameters and antimicrobial resistant bacteria with traditional treatments over one year. This technology is formed hanging sponge in an aerobic receptacle nitrifying column before it is passed to a similarly built anoxic, denitrifying column.

The DHS system had removal rates and lower overall effluent levels of Turbidity, tCOD, sCOD, and Total Phosphate (>80% removal) than the Imhoff and aerated systems, and it was able to match the performance of the large activated sludge plant for sCOD and BOD. The DHS removed greater levels

of sAmmonia than its rivals, particularly in the effluent levels, due to nitrification/denitrification. Most impressively, the DHS system was able to match or better the physio-chemical performance in bacterial testing i.e., it removed greater levels of coliforms, ESBL resistant bacteria, as well as vancomycin resistant bacteria than the traditional treatments. We also aim to undertake high-throughput qPCR and 16S DNA sequencing in time for the conference dates to determine AMR gene levels in influent and effluent for each wastewater treatment.

This study is currently running but the early data Down-flow hanging sponge (DHS) system compared with traditional wastewater treatments in Malaysia. Removes greater levels of COD, BOD, and phosphate than traditional treatments and removes greater levels of coliforms, ESBL, and vancomycin resistant bacteria.

**P 189      Quantitative microbial risk assessment of antibiotic resistant opportunistic pathogens in recreational surface waters in Singapore**

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In the last decade, the presence of antibiotic residues and antibiotic resistant bacteria (ARB) has been reported in various aquatic compartments, including hospital and domestic wastewater, surface water, ground water, sea water and even drinking water. Exposure of humans to ARB in the aquatic environment may occur through accidental ingestion of feacally contaminated water through recreation. Estimation of the probability of infection (Pi) caused by bacterial pathogens in water can be achieved through quantitative microbial risk assessment (QMRA). The purpose of this study aimed at assessing the public health risk associated with exposure to opportunistic bacterial pathogens and resistant ones through primary (swimming) and secondary (e.g. boating, canoeing, fishing, playing with water) recreational activities, although no swimming activities are allowed in any freshwater bodies in Singapore. Concentrations of *Escherichia coli*, *Enterococcus* and *Pseudomonas aeruginosa*, as well as the percentage of these bacteria resistant to several specific antibiotics in 4 water bodies and 2 tributaries were measured over a period of 15 months. The beta-Poisson dose-response model and the exponential model were used to measure the probability of gastro-intestinal illness (GI) with *Escherichia coli* and *Enterococcus*, as well as with the same pathogens resistant to specific antibiotics. The risk potential was evaluated using the 1986-U.S. EPA Ambient Water Quality Criterion that relies on *E. coli* and *Enterococcus* indicator organisms and limits the acceptable level of GI illnesses to 36 illnesses per 1000 swimmers per day (U.S. EPA, 1986, 2004). The mean probability of GI from total and resistant *Escherichia coli* for all recreational activities were almost all below the accepted threshold. The mean Pi with *Enterococcus* exceeded the threshold (5%-33%) for all recreational activities in tributaries, whereas the mean Pi with resistant *Enterococcus* exceeded the accepted threshold (0.01%-15%) for swimming in tributaries only.

**P 190      Impact of activated sludge based sewage treatment plant in the proliferation of antibiotic resistant bacteria**

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Widespread use and significant availability of antibiotics (AB's) in the environment led bacterial adaptability to AB's. Even at low concentrations (ng/L), long term exposure of AB's to the microbe rich environment could create a selective pressure on bacteria for resistance development. Sewage treatment plants (STP) are one of the places where favorable conditions are made available for bacteria to grow and degrade the organic content at faster rates. Un-metabolized AB's from the human body reach STP's through wastewater, this could expose bacteria to sub inhibitory levels of AB's in the STP's. The objective of this study is to identify the presence of antibiotic resistant bacteria (ARB) in a STP and to determine the effect of activated sludge based treatment on the prevalence of ARB. With

this aim, samples were collected from three different points of STP (Bio-inlet, Bio-outlet and Final effluent). Primary AB's used to isolate the resistant species are fluoroquinolones (FQs) and the heterotrophic plate counts ranged from  $5.91 \times 10^4$  to  $1.4 \times 10^2$  in the Bio-inlet and the final effluents for the three FQs (NOR, CIP,OFL). Antibiotic susceptibility tests were performed for 16 selected AB's. Total 120 resistant bacteria were isolated from the samples and high resistance is observed in GEN (95%) S (88%) TOB (85%) and K(76%). MAR index ranged from 0.43 to 0.81 throughout the treatment plant which means no single bacteria was resistant to less than seven antibiotics. There was a selective increase of FQ resistant bacteria (CFU/mL) over total bacteria after biological treatment. Furthermore, percent increase of antibiotic resistance is observed after activated sludge treatment for several antibiotics tested, for example LEV from 95% to 100%, GEN 85% to 100% and AK 42.5% to 77.5%. In addition, to understand the resistance species distribution at different sampling sites isolated bacteria were identified as belonged to enterobactereaceae family (*Escherichia*, *Salmonella*, *Shigella*) by 16s rRNA sequencing.

**P 191 Occurrence and fate of antibiotic resistance genes in wastewater treatment plants:  
Pond system in Namibia vs. activated sludge system in Germany**

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We compared the antibiotic resistance genes (ARGs) profile found in a Namibian pond system based wastewater treatment plant (WWTP) against an activated sludge system (AS) based WWTP in Germany. In Namibia, pond systems are often used for wastewater treatment due to the cost component and operational requirements, whereas, in a developed country like Germany a wide variety of technologies are implemented, the AS system being the most popular. The use of antibiotics for disease treatment in humans and livestock also vary between Germany and Namibia, extensively. A metagenomic approach was used to investigate wide-spectrum profiles of antibiotic resistance genes in wastewater and effluent of the WWTP in Namibia as well as in Germany. The abundant ARGs found in Germany were associated with aminoglycoside, multidrug, rifamycin, macrolide-lincosamide-streptogramin (MLS), and tetracycline; in Namibia with aminoglycoside,  $\beta$ -lactam, trimethoprim, and quinolone. The abundant ARG types found in Namibia and Germany differed, especially for multidrug resistance genes and ARGs associated with veterinary antibiotics. Amongst the top twenty most abundant ARG types found at each location, the abundance of ten ARG types increased in the effluent of the pond and AS system, respectively. Comparing the efficiency of each WWTP for clinically relevant ARGs, we found that the ARG reduction capacity of each WWTP depends upon the type of ARG. For example, the abundance of the aminoglycoside (*aph(6)*, *aph(3)*) and quinolone (*qnrS* and *qnrD*) resistance genes reduced in the pond effluent but increased in AS effluent, whereas, the abundance of MLS resistance genes (*ermB*, *ermF*) decreased in the AS but increased in the pond system. The analysis showed that the ARGs spectrum in Namibia is different than in German wastewater but requires attention due to often direct contact with the water (animals) and water reuse.

**P 192 Carbapenem resistant *E. coli* in treated wastewater from selected African cities**

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Wastewater treatment plants are considered as hotspots for antibiotic resistance development and dissemination through the release of effluents containing resistant microorganisms. This abstract therefore presents the concentration of carbapenem resistant *E. coli* in treated wastewater from selected African cities. This is part of a large pan-African project focused on the use of sewage analysis as a surveillance tool for antibiotic resistance.

Composite samples of 1 liter were taken from the final effluents of wastewater treatment plants serving at least 10,000 individuals in the cities of Dakar (Senegal), Monrovia (Liberia), Kumasi (Ghana), Port Harcourt (Nigeria) and Yaoundé (Cameroun) were taken. Serial dilutions of the samples were prepared and inoculated (100µl) unto chromogenic ECC plates (total *E. coli*), ECC-meropenem-cloxacillin-ZnSO<sub>4</sub> plates (carbapenemase-producers) and ChromID-OXA48 (**OXA-48** type Carbapenemase-Producers) and incubated at 37°C for 24 h.

Total *E.coli* concentrations in the treated wastewater was beyond wastewater discharge standards and out of the five cities, carbapenem resistant *E. coli* was detected in effluents from two. However, there were no *OXA-48* type of carbapenem resistant *E. coli* in Liberia. Although carbapenem resistant *E. coli* was not detected in treated wastewater from three of the countries, it does not eliminate the occurrence of other types of antibiotic resistant *E.coli* or bacteria in general. This therefore raises serious concerns for wastewater reuse which was observed or reported in Dakar, Monrovia and Kumasi.

#### **P 193 Clinically relevant β-lactamase genes in an urban river in South Africa**

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β-lactam antibiotics belong to one of the oldest and most prescribed classes of antibiotic that are used for managing infectious diseases. *AmpC* β-lactamase are known to inactivate a diverse array of β-lactam antibiotics, thus their presence in the environment can potentially lead to increased resistance against these antibiotics and would result in therapeutic failure. The aim of this study was to determine the prevalence of plasmid-mediated *AmpC* β-lactamase genes and integrase (*intI1*) in a rural-urban river system as well as the levels of various *AmpC* β-lactamase genes across. Water samples were collected from Mooi River catchment (2015-2016) and subjected to membrane filtration to obtain environmental DNA. Environmental DNA was extracted using the DNeasy® PowerWater® Kit (Qiagen). The detection of *intI1* and plasmid-mediated *AmpC* β-lactamase (MOX-, CMY-, DHA-, ACC-, ACT-, FOX-type) genes was conducted using conventional PCR. The *intI1* was present across all the sites that contained two or more of the *AmpC* variants. FOX-type and ACC-type β-lactamase were present across all the sites, they increased in levels from the rural sites M3 (Boskop Dam) to the urban sites (M8 Viljoen bridge). Real-time quantitative PCR revealed that copy numbers varied across the sites in the Mooi River with a range from 2.86x10<sup>-3</sup> to 8.01x10<sup>3</sup> copies/ng DNA. The MOX-, CMY- and ACT-type variants were only detected in one of the sampling periods at urban sites (Thabo Mbeki bridge, Trim park bridge and Pedestrian bridge). All of the sites impacted by urban run-off. This demonstrated that there is an anthropogenic impact which play a role in the proliferation of these resistant genes in the Mooi River catchment. The prevalence of *AmpC* β-lactamase in the Mooi River is a cause for concern, more especially since the water is for agricultural, recreational and religious purposes down-stream from the urban area. These genes could be incorporated in the human and animal microbiota thus increasing resistance to β-lactam antibiotics.

#### **P 194 The effects of pharmaceuticals and personal care products exposure on the behavior of *Stenotrophomonas maltophilia* isolated from drinking water**

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The presence of pharmaceuticals in water sources, including in drinking water (DW), is increasingly being recognized as an emerging and global concern for the environment and public health. Based on the principles of the "One Health" initiative, the present work aims to understand the effects of several

pharmaceutical and personal care products (PPCPs) on the behavior of a *Stenotrophomonas maltophilia* strain isolated from drinking water (DW). Eight PPCPs were studied individually and in combination: antipyrine, diclofenac sodium salt, ibuprofen (IBP), galaxolide, tonalide, carbamazepine (CBZ), clofibrate acid (CA) and tulosin. *S. maltophilia* biofilms were exposed for 4 weeks to all the PPCPs at trace concentrations detected in DW and concentrations 100× higher. Results did not evidence remarkable effects of PPCPs on planktonic *S. maltophilia* tolerance to sodium hypochlorite (NaOCl) and to antibiotics. However, *S. maltophilia* biofilm production and biofilm susceptibility to NaOCl was affected by PPCPs. Most of the significant alterations observed were caused by the combination of different ECs (CA+CBZ, CA+IBP, CA+CBZ+IBP). CA was the single compound present in all the combinations that altered bacterial behavior, increasing the ability of *S. maltophilia* to form biofilms and/or increasing biofilms tolerance to NaOCl. Therefore, *S. maltophilia* biofilms were exposed to CA for a longer period (12 weeks) and the effects on tolerance to chlorine and antibiotics were assessed as well as the impact on *S. maltophilia* virulence. CA caused no effects on biofilm formation, motility and siderophores production. However, biofilms formed after CA exposure were more tolerant to chlorine disinfection. CA also caused an increase on *S. maltophilia* tolerance to erythromycin. On the other hand, CA exposure slightly reduced *S. maltophilia* ability to internalize human colonic epithelial cells. In conclusion, results demonstrated that non-antibiotic contaminants may have important impact on water microbiome behavior, particularly on microbial tolerance to chlorine and antibiotics.

#### P 195      Antibiotic resistant bacteria in therapy pools, hotel pools and spa facilities

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Emerging and increasing antibiotic microbial resistance (AMR) represents one major threat to human health in Europe and worldwide. The major drivers behind the occurrence and spread of AMR are the use of antimicrobial agents and the transmission of antibiotic resistant microorganisms. Transmission pathways are not well understood and especially the role of the environment is not clear.

Because transmission of antibiotic resistant bacteria is feared patients in health care facilities are excluded frequently from important therapies. Beneficial therapies like hydrotherapy may be a source of infection, because it induces a larger release of bacteria into the immediate environment of patient and therapist. Data from the literature in other countries suggest that the existing water disinfection is not always sufficient to kill all released bacteria. Bacterial contamination can occur furthermore due to colonization of the pool equipment.

Another possible point of transmission are hotel pools and associated spa facilities where many people from very different areas mix. Especially hotels visited by guests from countries where antibiotic resistance is particularly prevalent, e.g. guests from the Arab world or the Middle East, but also guests from the Mediterranean are of interest.

Pools and their surroundings were sampled accordingly and were analysed for the occurrence of antibiotic resistant bacteria. Typical water quality parameters were determined. Additionally technical and operational details were recorded with a standardized questionnaire.

Antibiotic resistant bacteria were isolated in the pool water and more often in the pool environment. Operational difficulties, high bathers load and poor management of cleaning equipment were possible explanations for the observed contamination.

**P 196      Antibiotic resistance, virulence factors and genetics of *Clostridium perfringens* from surface water in South Africa**

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*Clostridium perfringens* produces a variety of toxins and enzymes that are responsible for severe diseases, such as gas gangrene. Numerous research exists on *C. perfringens* in clinical settings, however, its pathogenicity has been greatly neglected in environmental research. The aim was to determine the pathogenicity of environmental *C. perfringens* isolates by investigating antibiotic resistance and virulence associated genome content. *Clostridium perfringens* were isolated from surface water (river system) in the North West province of South Africa, and subjected to whole genome sequencing on an Illumina MiSeq. Gene prediction and annotation of genomes were preformed using various bioinformatics tools. *In silico* analysis of three *C. perfringens* strains showed draft genomes with a total length of between 3.14 - 3.52 Mbp and GC content of 28.18%. A total of 3,111 protein coding regions were identified on average, which included 8 to 10 rRNAs, 91 to 93 tRNAs and 1 tmRNA gene in each genome. The genomes harboured several antibiotic resistance genes for macrolide-lincosamide-streptogramin, β-lactam, trimethoprim, tetracycline, kasugamycin and bacitracin. They also revealed the presence genes encoding for glycopeptides and multidrug resistant efflux pumps. In addition, 13 antibiotic resistance islands were found in all three strains. Further analysis showed 35 virulence genes that encode for hemolysins, enterotoxins, sialidase, hyaluronidase, collagenase, perfringolysin O and alpha-clostripain. The genome assembly also revealed the presence of two members of the double component VirR/VirS regulon. Genomic comparison with *C. perfringens* strain 13 using orthologous average nucleotide identity resulted in values of between 98.50% and 98.52%. Therefore, these three *C. perfringens* strains can be classified as type A strains, which are human pathogens.

**P 197      Characterisation of Antimicrobial Resistance in a Wastewater Treatment Plant**

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Antimicrobial resistance (AMR) is considered one of the universal threats to humans, animals and the environment. The latter has been recognised only recently as a relevant component which may contribute to the spreading of AMR, for example through waterbodies. Indeed, water (fresh and marine waters) can act as reservoir of antibiotic resistant bacteria (ARB) due to a combination of naturally occurring resistance with resistance present in animal and human waste. The release of antibiotics in water determines a constant pressure to which microorganisms are exposed thus causing persistence of antimicrobial resistance genes (ARG) and facilitating the spread of the acquired resistance from environmental microbes to human or animal pathogens.

Wastewater treatment plants (WWTP) are among the main sources of antibiotics and resistance-carrying genes in the environment. Antibiotics are only partially removed during the different treatment phases in the WWTP and once released into the environment, they can be easily degraded or can persist and therefore accumulate. In our studies we have investigated the water entering a WWTP from a large urban area, at the outcome (effluent) and at a location 5km downstream the WWTP. The concentration of a large panel of antibiotics has been determined and the expression of AMR has been examined by sequencing water collected samples. In addition, the microbial community composition was characterised by 16S metagenomic analysis. The final goal of this project is to determine the efficiency regarding the removal, or possibly even accumulation, of antibiotics and ARG in a WWTP and determine the dilution or concentration factor for the downstream freshwater sampling site.

**P 198      Resistance to disinfection agents: Adaptation of *P. aeruginosa* to sub-inhibitory concentrations of quaternary ammonium compounds (QACs)**

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Quaternary ammonium compounds (QACs) are among the most used disinfection agents in consumer products and in industrial applications. Because of their poor biodegradability, after use, they will persist in technical and natural aquatic systems at sub-inhibitory concentrations. Implications of QACs on the development of antimicrobial resistance was detected in several studies, however, the effect of a constant sub-inhibitory concentration on the development of bacterial resistance remains unknown.

The aim of this study was to determine if constant sub-inhibitory concentrations of benzalkonium chloride (BAC) and cetyltrimethylammonium chloride (CTMA) are able to promote a stable adaptation of populations of *P. aeruginosa*. Additionally, the study investigated cross-resistances between QACs and with antibiotics and nanomechanical properties of the membrane using atomic force microscopy (AFM) were explored.

The exposure to sub-inhibitory concentrations of QACs led to a maximal but reversible increase of the minimum inhibitory concentration (MIC) by a factor 1.45 for CTMA and a maximal irreversible increase of the MIC by a factor 1.9 for BAC. Cross-resistance was observed with the QACs but not with antibiotics: bacteria exposed to CTMA had an increased MIC for BAC of a factor 1.8 and bacteria exposed to BAC had an increased MIC for CTMA of a factor 1.75. Analyses of the membrane by AFM showed increase in the cell's roughness and stiffness after treatment with QACs.

In populations of *P. aeruginosa*, constant sub-inhibitory concentrations of QACs were able to promote adaptations mechanisms, which were irreversible in the case of BAC. Furthermore, cross-resistance between the selected QACs were observed. Since low sub-inhibitory concentrations of QACs can be detected in natural and technical aquatic systems, this may lead to a dissemination of bacteria with higher QAC resistance or with QAC multiresistance, which can be a threat to the urban water management.

**P 199 Risk assessment of intra-urban dissemination of multidrug-resistant bacteria in a wastewater system in the Ruhr Metropolis (Germany)**

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Water compartments occupy a pivotal role for the dissemination of antibiotic resistance (ABR) elements. In particular wastewater has been identified in various studies as a hotspot. The majority of studies investigate the contribution of ABR elements of the human inpatient sector only, thereby focusing on end-of-pipe analyses. Currently, there is only limited research on the contribution of the human outpatient sector albeit antibiotic use is much higher in this domain in most countries worldwide.

This study examines structural facility and “socio-spatial” hotspots of antibiotic use utilizing World Health Organization’s Sanitation Safety Planning approach as a basis. This approach allows for a hazard analysis and the identification of critical control points within a wastewater system. It will be tailored to a single microbiological hazard, the discharge of multidrug-resistant bacteria.

Following the concept of the social gradient of health, whereby social and health status are positively associated, socio-spatially distinct districts in the city of Dortmund in the Ruhr Metropolis, Germany, have been selected for sampling. It is analysed whether differences in antibiotic use can be identified within and between these spatial entities based on socio-economic status and if such differences are reflected in the concentration of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) municipal wastewater of the spatial clusters. In addition, structural facility hotspots (e.g. hospitals, elderly care facilities, etc.) will be identified and mapped. ESBL-producing *E. coli* are used as the indicator organism due to the great importance of beta lactam antibiotics in outpatient care, accounting for approximately 43% of all antibiotic prescriptions in Germany in 2014.

Here we present the overall concept of the risk assessment and preliminary findings of the on-going investigation on whether and how human outpatient antibiotic consumption influences the occurrence of ESBL-producing *E. coli* in the municipal wastewater system of the city of Dortmund, Germany.

**P 200 Presence of Antibiotic-Resistant Bacteria (*Escherichia coli*) in River Water: first Survey in Wallonia (Belgium)**

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Antibiotic resistance is recognized by the World Health Organization to be one of the greatest threats to global health. *Escherichia coli* (or *E.coli*), a gram-negative bacillus that belong to the coliform group, is present in the intestinal tract of warm-blooded animals. It is released in the environment through their faeces. *E.coli* is thus usually used as an indicator of faecal contamination and water quality. The presence of enteric bacteria in aquatic environments represents a problem of public health, especially due to the fact that such bacteria can carry resistances to antibiotics. Aquatic systems can then become a vector of spreading of antibiotic resistance through the environment.

In this study, a survey of the presence of antibiotic-resistant *E.coli* is conducted in a river in eastern Belgium, with the objective to identify the potential sources of antibiotic-resistant bacteria.

Antibiotic selection was based on reported consumption in hospitals and sales in pharmacies. Selection also aimed at covering as many antibiotic families as possible, as well as all areas of use (veterinary, hospital, and mixed use). Twelve EUCAST-referenced substances were selected. *E.coli* ATCC 25922 was used as reference strain. The agar disk-diffusion method on Mueller-Hinton agar was used to study antibiotic-resistance based on the EUCAST guidelines.

The study focuses on the Ourthe watershed, in Eastern Wallonia. The Ourthe river is characterized by two branches (Eastern and Western Ourthe) which join in Engreux. Both branches have their spring in the province of Luxembourg, in a rural area. The Ourthe river is 181 km long and flows into the Meuse river in Liège, in a heavily urbanized environment. This variety of landscapes motivated the focus on this watershed: samples collected at different locations along the river will allow the selective differentiation of the sources of antibiotic-resistant bacteria in the river. Two sampling campaigns are planned, in order to identify a potential seasonal effect.

