VERBAL PRESENTATIONS
A systematic review on environmental exposure to antibiotic resistance bacteria and its impact on human health with emphasis on methodology for determining linkage between exposure and health outcomes

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Introduction: It is well documented that there is considerable overuse, careless use, inappropriate use and unregulated availability and use of many antibiotics in both medicine and veterinary medicine, as well as extensive and largely unregulated use in animal agriculture and aquaculture, including for animal and fish growth promotion. These imprudent uses and abuses of antibiotics contribute to the extensive presence of antibiotic residues, their metabolites, multiply antibiotic resistant bacteria and their functional genes in human and animal wastes and in fecally and otherwise antibiotic-contaminated water, soil, sediments and in water-dependent food crops such as seafood and produce. Despite the breadth of consequences, the extent of the risk to human health is unknown because methods used to link environmental exposure to human health outcomes disparate and weak. The purpose of the review is to evaluate environmental exposure to antibiotic resistant bacteria (ARB) and human health outcomes through evaluating the methods used to create the linkages, identify gaps in methodologies, and make recommendations for establishing stronger linkages between antibiotic resistant bacteria in the environmental and associated with human health outcomes. Methods: A systematic review protocol was developed a priori and pretested prior to implementation. A pretested search strategy tailored specifically to the purpose of the study was developed and implemented in the following databases: PubMed and Web of Science. The search strategy consisted of the following categories: antibiotic, resistance, microbes, environmental reservoirs, and health impact. A data extraction protocol was developed a priori, and pretested. Data was formatted and analyzed descriptively (frequency and percentages) to allow assessment of methods used to create environmental linkages between transmission of ARB in environmental and human reservoirs to human health outcomes to identify gaps, and make recommendations for establishing stronger evidence for links between environmental exposure to ARBs and adverse human health outcomes Results and Discussion: Using systematic review methodology, relevant literature was identified and characterized to explore human health outcomes associated with environmental exposure to antibiotic resistant bacteria. The most frequently investigated studies focused on associations between ARB in environmental media and animals compared to ARB found in humans rather than epidemiological studies that characterized direct exposure to ARB and associated human health outcomes. Studies evaluated in this review were evaluated based on sampling methodology, microbiological methodology (phenotypic and/or genotypic), and degree of relationship between non-human and human samples. Overall, there is lack of consistency in methodologies, and consideration for human health outcomes when investigating environmental media by various health-related environmental stakeholders concerned with water, sanitation and hygiene.

An Evaluation of E. coli Quantification in Compartment Bag Tests (CBT) Incubated at Multiple Temperatures

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Escherichia coli (E. coli) is used as an indicator of fecal contamination and therefore serves as an indicator of the microbiologic quality of water. The World Health Organization (WHO) recommends that no E. coli should be detected in 100 mL of drinking water. While several quantitative E. coli tests have been developed for field use, they often require expensive equipment and up to 24-hour access to electricity. Low-cost, simple E. coli quantification tests are needed to assess drinking water quality in low-resource settings. The Compartment Bag Test (CBT) is an easy-to-use, portable test that quantifies E. coli in water. The test utilizes chromogenic E. coli broth culture medium and a most-probable-number (MPN) format to provide both detection and quantification of E. coli in 100 mL samples. The manufacturer states that the CBT is effective at incubation temperatures >25 °C, which would allow incubation at ambient temperatures common in tropical regions. Therefore, the CBT could eliminate the need for incubators and electricity and could vastly simplify water testing in low-resource settings. This study assessed the performance of the CBT incubated at multiple incubation temperatures and times. Both distilled water seeded with approximately 10 E. coli/100 mL and primary-settled wastewater diluted to approximately 10 E. coli/100 mL were processed using the CBT and incubated at 25, 30, and 35 °C. Compartments were scored for characteristic blue-green color formation at 24, 30, and 48 hours, according to manufacturer’s instructions for each temperature. Numbers of positive and negative compartments corresponded to an E. coli concentration in the CBT MPN table. IDEXX® Colilert®-18 and membrane filtration (MF) assays were completed in parallel. In seeded distilled water, geometric mean E. coli (MPN/100 mL) in CBT tests was 10.3 (95% confidence interval 5.7-18.5) when incubated at 35 °C for 24 hrs; 10.9 (5.4-22.2) when incubated at 30 °C for 30 hrs; and 7.4 (4.9-11.1) when incubated at 25°C for 48 hrs. Geometric mean E. coli for parallel IDEXX and MF controls was 5.7 (4.4-7.3) and 8.0 (6.2-9.0), respectively. In diluted wastewater, geometric mean E. coli (MPN/100 mL) in CBT tests was 5.7 (4.0-8.0), 7.1 (4.3-11.7), and 8.8 (4.7-16.4), respectively. Geometric mean E. coli for IDEXX and MF parallel controls was 8.0 (6.8-9.5) and 9.6 (8.5-10.9), respectively. Determining the microbiological quality of drinking water provides information about its safety and may lead to implementation of water treatment or disuse of contaminated sources, and ultimately, a reduction in waterborne disease. Low-cost, simple tests that eliminate the need for electricity, cold chain, and specialized equipment and technicians may provide an advantage to standard tests in low-resource settings. Our data suggest that the CBT, when incubated at manufacturer-recommended temperatures and times, provide E. coli concentration estimates consistent with those of standard tests. Additional studies on the effect of temperature variability are warranted.

**Human, wildlife, and environmental drivers of water quality and health in Chobe Botswana**

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With nearly 50% of the Earth’s surface characterized as 'dryland', the linked issue of water quality, health and water scarcity is identified as an urgent global problem but particularly in dryland countries such as Botswana. Our work on water quality-health interactions in the Chobe River region of Northern Botswana identifies important linkages between human and wildlife health, landscape change, and
degradation in ecosystem services related to water quality and sanitation deficiencies. Our preliminary analysis of diarrheal disease in children under five years of age (2006-2013) in Chobe District indicates that the scope of diarrheal outbreaks covary significantly with declines in water quality and the magnitude of hydrological events and other meteorological factors. We present spatial patterns of Shiga toxin-producing Escherichia coli (STE C) and human specific Bacteriodes within the Chobe River and compare this to the temporal and spatial patterns of water quality dynamics. We discuss the potential influence of wildlife and human health behaviors on these observed patterns. Couplings between the environment and human health are complex and dynamic. Multiple interdependent drivers are involved including environmental, hydrological, and meteorological factors, temporal and spatial patterns of wildlife population dynamics, and human health behaviors and traditional belief systems.

Microbial Indicators and Pathogens in Raw Sewage, Highly Treated Reclaimed Water, and Sewage Impacted Surface Waters in North Carolina: Dilemmas in Estimating Health Risks from Recreational, Agricultural and Drinking Water Use

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Treated wastewater is discharged routinely to ambient surface waters that are used downstream for primary contact recreation or as a sources of agricultural or drinking water. Use of discharged wastewater for either non-potable purposes such as agriculture or as source water for water supplies, is increasing both unplanned, where upstream wastewater discharges reach water supply intakes, or purposefully, with treatment by engineered water reuse systems. The microbial quality of these water sources is of concern, particularly with regard to pathogen concentrations in both reclaimed water and wastewater-impacted surface water sources of recreational, agricultural and drinking water impacted by sewage discharges. To address this concern, indicators and pathogens representing the three classes of microorganisms, bacteria, viruses and protozoans, were quantified in samples of: (1) raw sewage, (2) tertiary treated, dual disinfected (free chlorine and UV radiation) reclaimed water, and (3) sewage impacted surface waters used for recreation or as a water supply source in the Research Triangle area of North Carolina. Results to date indicate that the treatment processes required to produce high quality reclaimed water effectively reduce indicator bacteria, viruses, and protozoan parasite surrogates to low concentrations (≤3 E. coli/100 mL, ≤5 coliphages/100 mL and ≤5 Clostridium perfringens/100 mL) that meet requirements for agricultural and drinking water use as well as meeting bacteriological requirements for recreational water. However, human enteric virus pathogens (adenoviruses and noroviruses) and protozoan parasites (Giardia and Cryptosporidium) were still detectable in 10-liter volumes of many reclaimed water samples by nucleic acid-based (RT-)PCR and immunofluorescent microscopic methods, respectively. The extent to which these human enteric viruses or protozoan parasites are actually infectious has not yet been determined due to lack of reliable methods, technical demands and high cost. Based on NC regulations, reductions of indicator bacteria, viruses and protozoan parasites from raw sewage by tertiary treatment followed dual disinfection were satisfactory at 6, 5 and 4 log10, respectively. Despite this, average log10 reductions of pathogens, especially human enteric viruses and protozoan parasites, were not as great, were highly variable and did not meet State log10 performance targets for high quality reclaimed water. Analysis to
date of sewage impacted surface water samples near intakes for drinking water supplies indicates that dual disinfected reclaimed water is of higher microbial quality than such surface waters and that surface waters do not consistently meet bacteriological quality requirements for recreational water. As log10 reductions of human enteric pathogens were less than reductions in fecal indicator organisms, important questions remain regarding the infectivity of these organisms, and the suitability of using (RT)-PCR and immunofluorescent microscopy methods for pathogen detection in treated wastewater samples. For reliable management decisions intended to protect public health, it is important to not only determine pathogen concentrations in samples, but also their potential for infection and illness from exposures. Such data on culturability and infectivity of detected pathogens are often not obtained, leading to great uncertainty about their human health risks.

**Eco-genomics of Temperature Effects on Partial Nitritiation/Anammox (PN/A) Reactor**

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Sha Wu; B Dutilh; Ramesh Goel

Background: Nitrogen is regarded as one of the main nutrients of concern in municipal waste-water, storm water, agricultural runoff and in many ecosystems such as in streams and wetlands. Release of excessive nitrogen into to the environment acts as a major cause for eutrophication in surface waters. Managing nitrogen in ecosystem using engineered system has been inducted as one of the 14 grand challenges by National Academy of Engineers [1]. Hence, engineers are constantly looking for alternative processes to efficiently mitigate nitrogen in waste-waters. Partial nitritation and anammox (PN/A) process combined together brought in an innovative biotechnological revolution for energy-efficiency with reduced greenhouse gas emission during N removal from waste-water. PN process provides nitrite, which acts mainly as a terminal electron acceptor for oxidation of ammonia to Nitrogen gas through Anammox (AMX) process. PN/A reactors are generally maintained at mesophilic temperatures, despite the fact that AMX bacteria in natural ecosystems have been found to thrive below 10°C. Arguing, the possibility of PN-AMX process is metabolically active even at low temperatures. In this study, we investigated the temperature-effects by subjecting a pilot scale PN/A reactor to different gradients, as metabolic pathways underlying remains poorly understood. Methods: Metagenome and meta-transcriptomes were generated from the biomass obtained from PN/A reactor to gain insight into the key interactions between prokaryotes involved. The 9-L batch reactor was fed with reject water from anaerobically digested sludge supernatant. To investigate the effect of temperature on the PN/A metabolism, it was exposed to temperature gradients of 35°C, 21°C and 13°C. DNA was extracted from collected biomass of PN/A reactor at 35 °C. RNA was extracted at each gradient post acclimatization period to generate high throughput sequencing data. Briefly, DNA sequences were quality trimmed, phylogenetically classified using PhyloSift [2] and assembled into contigs using Omega [3] and subsequently binned into genomes using MaxBin [4] based on differential coverage and tetra-nucleotide frequencies. RNA-Seq data were assembled into de-novo transcripts using trinity [5] followed by abundance estimation and differential expression of de-novo transcripts. Extraction of the coding regions was performed using TransDecoder [5] followed by annotation using Trinotate [5]. Results: A total of 34 bins were obtained from the metagenome among which, 18 were partial to near-complete genomes. 3 species were dominant in redundancy, Planctomycetes sp., Anaerolinea sp. and Ignavibacterium sp., which were estimated to be >90% complete based on single-

**Ebola Virus and Surrogate Persistence and Disinfection in Wastewater**

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In March 2014, an unprecedented outbreak of Ebola virus began in Western Africa. In response to the Ebola epidemic, both the World Health Organization and the United States Centers for Disease Control advised direct disposal of Ebola-contaminated wastewater into sewage systems and latrines without disinfection. In the wake of this recommendation, questions were raised regarding appropriate handling of Ebola virus contaminated wastewater. In response to these unknowns, we have conducted research investigating appropriate surrogate viruses for Ebola virus, Ebola virus persistence in wastewater, and Ebola virus disinfection. Surrogate testing suggested Phi6 to be the best performing surrogate evaluated; however, Phi6 persistence was not conservative under all conditions. Phi6 persistence under various environmental conditions will be presented, including the impact of biological activity on Phi6 persistence. In tests of Ebola virus persistence in sterilized wastewater, no virus was recovered from an initial viral titer of 10^2 TCID50/mL. From an initial viral titer of 10^6 TCID50/mL, the viral titer decreased approximately 99% within the first test day; however, the subsequent viral titer decrease was less rapid and infectious Ebola virus particles persisted for all eight days of the test. The inactivation constant (k) was determined to be -1.08 (2.1 days for 90% viral titer decrease). Wastewater composition, the necessity for wastewater sterilization, and testing at 20°C suggest this data to be conservative (i.e. a more rapid inactivation would be expected in-situ). Ongoing research into Ebola virus disinfection in wastewater using free chlorine (bleach) will also be presented. To conclude, implications from study findings and research needs to inform wastewater handling during future viral outbreaks will be discussed.
Quantification of Pathogenic Viruses at Two Popular Southern California Surfing Beaches Impacted by Stormwater Discharge

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Southern California waters are not impacted directly by sewage treatment discharge, as most sewage discharge occurs from deepwater ocean diffusers at least a few miles offshore. However, stormwater has major impacts on the water quality of southern California beaches, as evidenced by widespread utilization of 72-hour rain advisories for the region following measurable precipitation. The land use patterns and aging infrastructure contribute to the water quality degradation during wet weather. Failing sewage infrastructure in the form of aging clay pipes, corroded cast iron pipes, high impervious surface coverage, and high flows following even modest precipitation are prominent reasons that stormwater discharge in southern California poses serious concerns. A particular group of interest and concern are surfers, repeat visitors to the beach that stay in the water for elongated periods, and especially favor storm conditions that bring large waves. A large collaborative study was conducted to assess the impact of stormwater on surfer health, with a portion of the effort devoted to formal quantification of human viral pathogens of concern, such as human norovirus genotypes I (GI) and II (GII), human adenovirus, and human enterovirus. Sampling was conducted during the wet seasons of 2014/2015 and 2015/2016 and encompassed six different storms. The study was conducted with the use of digital droplet PCR, which offers the ability to quantify human viral pathogens at low concentration, while avoiding the detrimental impacts of inhibition observed previously for complex water matrices such as stormwater. Two stormwater discharges were assessed, Tourmaline Creek (TC) and San Diego River (SDR). Tourmaline Creek drains a small urban watershed and the San Diego River drains a large mixed urban/undeveloped watershed. FIB quantification was conducted to quantify total and fecal coliforms, E. coli, and Enterococcus sp. using traditional culture-based methods, with concentrations of E. coli and Enterococcus sp. reaching 61,310 and 50,000 MPN [or per 100 ml , respectively, in the discharge. Norovirus, adenovirus, and enterovirus quantification was conducted via digital droplet PCR with special attention to quality data return through the use of optimized processing approaches and quantification of RNA and DNA controls. Norovirus GII was detected in 86% of the discharge samples with concentrations ranging from non-detect to 495 gene copies per 100 ml, and Norovirus GI was only quantified in 10% of the samples. Adenovirus quantification was more sporadic than norovirus at the two locations, with quantification in 20% of samples, with concentrations ranging from non-detect to 42 gene copies per 100 ml. Enteroviruses were not detected in any of the stormwater discharge samples. Direct viral pathogen quantification via digital droplet PCR offers advantages over qPCR, because of the potential for heightened sensitivity, the ability to examine the reaction success, and lessened impact of PCR inhibition. Direct viral pathogen quantification data are valuable parameters to evaluate the performance of FIB and coliphage as indicators, and the potential risks to southern California surfers during storm conditions.

Development and evaluation of monitoring methods for surveillance of antimicrobial resistant fecal indicator bacteria in the environment: Leon, Nicaragua and Chapel Hill, North Carolina

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Background - The reduced efficacy of antimicrobials used to treat infectious diseases poses an enormous health challenge. To better understand the burden and potential exposure risks of antimicrobial resistant bacteria (ARB) and indicators for them, a surveillance system supported by uniform monitoring methods is needed for the direct detection and quantification of important ARB in clinical, community and environmental settings. To address this need, UNC Chapel Hill and UNAN-Leon are collaboratively investigating the development and use of an indicator system for gram-negative fecal indicator bacteria that produce Extended Spectrum Beta Lactamase (ESBL) as well as Klebsiella Pneumoniae Carbapenemase (KPC). The goals of this research are to 1) compare a clinical medium, CHROMagar Orientation agar, to a standard E. coli/cloiform medium, Bio-Rad Rapid E. coli 2 agar, to quantify these bacteria in wastewater and water, and 2) to evaluate CHROMagar media to detect ESBL and KPC resistant bacteria in clinical, community and environmental samples. Methods - Parallel samples of hospital sewage, raw municipal sewage, secondary effluent and surface waters upstream and downstream from the effluent discharge point are collected and analyzed using standard membrane filter methods on Bio-Rad Rapid E. coli 2 agar, CHROMagar Orientation, CHROMagar ESBL, and CHROMagar KPC media. Colony forming units (CFUs) were observed and recorded as discrete counts according to color guides provided by the manufacturer and concentrations were expressed as colony forming units (CFU)/100 mL. Samples were streaked to purity and isolated. Approximately 600 isolates of suspected ESBL and KPC E. coli were analyzed from Leon and Chapel Hill in combination with stool isolates from a study of healthy kids living in Leon near the sources of exposure. All samples were characterized by similar phenotypic and molecular methods (e.g. Kirby Bauer, Hodge, PCR and PhP) to determine their relatedness and clonality in these two geographically distinct regions. Results - Both sites find no statistically significant differences in E. coli and coliform detection and quantification between the standard environmental medium, Bio-Rad Rapid E. coli 2 agar and the clinical medium, CHROMagar Orientation for all samples, including hospital and municipal sewage and surface waters. High levels of ESBL and KPC resistance are being detected in E. coli and TC of hospital raw sewage. Higher levels of ESBL and KPC bacteria are seen in Chapel Hill Hospital than in Leon Hospital raw sewage. Lower but still appreciable levels of such resistant bacteria are detected in municipal raw sewage with even lower but still detectable levels occurring in environmental surface waters, with the exception of Leon, where very high levels of KPC resistant TC are observed. For both sites, the additional studies have positively confirmed over 90% of the identities and the antimicrobial resistance profiles of the E. coli and coliforms detected. In terms of clonality between ESBL E. coli from Leon and Chapel Hill, 32 common clones were found. Out of these 32 clones, 4 were clustering Chapel Hill and Leon isolates, of which 3 were recovered from Hospital, raw and secondary sewages. The other clone cluster isolates were from Hospital, raw and secondary treated sewage of Chapel Hill and Leon and from the stools of healthy kids of Leon. Conclusion - In summary, CHROMagar clinical media appear to be effective to directly quantify ESBL and KPC E. coli and coliform bacteria in environmental samples of water and wastewater by standard membrane filter methods. These media and methods have promise as a candidate indicator system to detect and quantify ARB of health concern in environmental media as a monitoring system to support environmental surveillance and during outbreaks. To our knowledge, this is the first report linking circulating environmental ARB isolates and those present in the gut microbiota of young healthy kids. Global spread of ARB merits evaluation across other geographic regions in US and abroad using parallel, matching methods to identify ARB threats and
detect outbreaks.

**Characterising Antibiotic Resistance in Singapore Water Bodies and Tributaries**

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The threats posed by antibiotic resistance bacteria (ARB) have detrimental effects on human health, as infections due to such multiple drug resistant bacteria are more complicated to treat and result in a higher mortality rate. Aquatic ecosystems can serve as hotspots for the accumulation of antibiotic compounds and for ARB to thrive and multiply and exposure to contaminated waters can result in infections that are difficult to treat with antibiotics. Carbapenem resistant Enterobacteriaceae (CRE) and Extended-spectrum beta-lactamase producing Enterobacteriaceae (ESBLs) are among the few of key players which pose a risk to antibiotic resistant infections in humans. In this study we determined the occurrence of 5 different opportunistic pathogens (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella spp. and Enterococcus spp.) which exhibit resistance to one of the six different types of antibiotics [Amikacin (AMI), Ciprofloxacin (CIP), Ceftazidime (CEF), Cotrimoxazole (COT), Meropenem (MER) and Vancomycin (VAN)]. We used selective agar supplemented with one of the 6 antibiotics at concentrations adhering to intermediate values, for each of the selected pathogens based on the 2015 Clinical and Laboratory Standards Institute (CLSI) performance standards for Antimicrobial Susceptibility Testing. This method yielded quantitative datasets (colony forming units/mL of sampled water) of viable ARB concentrations which was used as a tool to track fluxes of ARB targets in our local waterways. To identify spatial and temporal differences, water samples were collected from 4 water bodies and 4 tributaries in Singapore over different monsoon seasons. As a general observation, higher concentrations of ARB targets (~80 folds) were present in tributaries compared to water bodies. Preliminary data indicated an increase in ARB concentrations after rain events which could be attributed to surface runoffs. In particular, concentrations of antibiotic resistant K. pneumoniae and Salmonella spp. showed the most increment among all the ARB targets after rain events.

**Factors Influencing Point-of-Use Chlorine Disinfection Efficacy against a Model Fecal Indicator Bacterium in Water using Standard Performance Evaluation Testing Conditions**

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The evaluation of point-of-use (POU) household water treatment (HWT) technologies requires the consideration of multiple variables that impact performance and the microbial water quality of the treated water. The existing variability, diversity and lack of specification of POU technology performance evaluation testing specifications, materials, methods and conditions, creates a situation in which POU technology performance evaluations may very well produce test results that are widely different and highly variable. Chlorine disinfection has been widely used and accepted worldwide as an effective means for treating household drinking water. However, little research has been done to rigorously assess chlorine disinfection effectiveness at POU among different types of fecal indicator
bacteria, prepared under different conditions, and evaluated in different waters. Additionally, there are several types of quantal and enumerative assays to quantify indicator bacteria, but little has been done to characterize how these assays compare for analysis of bacteria in water treated at POU with chlorine. In this study, experiments assessed the disinfection efficacy of chlorine, as sodium hypochlorite, on Salmonella typhimurium and Raoultella terrigena, formerly Klebsiella terrigena, in different test waters. We compared a chemically defined, buffered test water spiked with lab strains as well as ambient surface water samples collected from the field. Bacteriological analyses using IDEXX Quantitrays, membrane filtration colony count methods, and multiple fermentation tube tests were compared for quantification of test bacteria. Results demonstrated that the time to achieve the chlorine disinfection 6 log10 reduction target varied greatly. Changes in conditions of test bacteria preparation greatly impacted microbial reductions by as much as 6 log10 CFU/100 mL when examining washed and unwashed cell preparation conditions. Different analytical methods to quantify the test bacteria gave results differing by as much as 7 log10 CFU/100 mL bacterial levels in the test waters when comparing IDEXX Quantitrays to membrane filtration colony counts. Changing the initial free chlorine concentration in test waters from 5.0 mg/L to 2.5 mg/L resulted in increased bacterial reduction variability, less efficacious disinfection by 2 log10 CFU/100 mL, and significantly reduced free chlorine residual. These results highlight important experimental factors that influence the results from standard performance evaluations of POU water treatments using chlorine, resulting in great differences in log10 bacteria reductions. These findings identify some key factors that require further study and standardization in order to better specify performance evaluation test conditions for reproducible POU chlorination efficacy testing. Such studies will provide a basis for improved assessment methodologies for POU performance evaluation and has direct relevance for the identification of effective HWT technologies that may then be approved or certified.

Metagenomics approach for comparative study of microbial community structure in granular and conventional activated sludge

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Background Conventional wastewater treatment (CWT) process has long been employed for biological removal of nitrogen, phosphorous and dissolved organics (rbCOD). Aerobic granular sludge technology (AGT) is an up-and-coming advancement towards conventional wastewater treatment process. It has an edge in terms of robustness, nutrient removal (N and P), lesser plant footprint requirement, and excellent settling characteristics (sludge separation). Aerobic granules are microbial aggregates without requirement of any carrier material. Due to alternating layers of nitrifying, denitrifying and Polyphosphate Accumulating Organisms (PAOs) bacterial communities, it is capable of complete removal of nitrogen, phosphorous and dissolved organic carbon (rbCOD). Hence, metagenomics study can help achieve a better understanding towards the enormous biodiversity in both the systems and providing a clear reasoning for differences in microbial composition responsible for distinctive processes taking place. Methodology A 2.5 L reactor was set to cultivate granules (AGT), using activated sludge as seed and hydraulic retention time (HRT) of 8 hours and settling time of 1.5 minutes. Simultaneously a 2 L reactor was set to enrich activated sludge flocs (CWT) with hydraulic retention time of 12 hours and settling time of 50 minutes. Synthetic wastewater consisting of ammonia,
phosphate, acetate and trace elements were used as a feed for both the reactors. Biomass from AGT and CWT lab-scale reactors was collected to extract genomic DNA and one microgram of was submitted for high throughput sequencing on an Illumina HiSeq DNA sequencer (Illumina, California, USA) to generate 125 nt paired end reads. Pre-assembly analysis included Quality trimming using sickle version 1.33 [2] and phylogenetic analysis using Phylosift [1]. The reads were assembled using Omega [3] and subsequently binned using MaxBin [4] based on differential coverage and tetranucleotide frequency. Results A total of 39 and 28 bins were recovered form AGT and CWT respectively, 5 of which were estimated to be >80% complete based on single-copy gene analysis in case of CWT whereas, in case of AGT, 7 bins were extracted. Phylogenetic assessment revealed a dominance of Thauera sp., Candidatus Accumulibacter Phosphatis sp. clade II A, Chitinophagaceae sp., incase of CWT biomass whereas AGT biomass revealed a dominance of Rhodocyclaceae sp., Candidatus Accumulibacter Phosphatis sp. clade IIA (PAOs), Chitinophagaceae sp., Nitrosomonas sp., Xanthomonadaceae and Saprospiraceae sp. Conclusion Presence of Candidatus Accumulibacter Phosphatis sp. in both systems explained the phosphorous removal-taking place in both the systems. Nitrification was explained by the presence of Nitrosomonas sp. in the AGT reactor. These findings are capable of providing an insight on to the various microbial interactions taking place in both the systems. Further analysis would provide an in depth insight into the novel metabolic network.


A Systematic Review and Meta-Analysis of Observational Studies Evaluating the Association Between Recreational Exposure to Ambient Waters and Adverse Health Outcomes

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Background: Numerous epidemiological studies have examined the association between ambient water quality during recreational activities and health outcomes, some of which supported the development of EPA's 2012 Recreational Water Quality Criteria. Most of these studies examined primary contact recreational activities where exposure to ambient water usually involves accidental ingestion and/or complete immersion (such as swimming and surfing). Fewer studies examined secondary contact recreational activities where exposure to ambient water is typically incidental or unintended (such as boating and fishing). Reduced exposure to ambient water during secondary contact activities may result in a smaller risk of illness compared to primary contact activities. However, there is no scientific consensus that the risk of illness associated with primary and secondary contact recreational activities are different. Objectives: Perform a systematic review and meta-analysis to evaluate the current scientific evidence for a difference in the risk of illness associated with primary contact and secondary contact recreational activities in ambient water. Methods: A protocol outlining the steps for the systematic review and meta-analysis was developed and peer-reviewed. A systematic
literature search of five databases was performed to identify studies published on or after the year 1950 examining associations between recreational exposure to ambient water and health outcomes. Reference lists of relevant studies were also manually searched. Meta-analyses were conducted for studies reporting suitable measures of risk (odds ratios or relative risks provided or easily calculated from data in the study). Study quality, heterogeneity and publication bias were also assessed. Results: The systematic review identified 473 studies, providing a total of 76 observational studies for inclusion in the meta-analyses. The studies exhibited substantial heterogeneity with respect to study design, evaluated health outcomes, exposures descriptors, water quality, and age of study subjects. A simplification framework was developed and documented to address this heterogeneity and allow meta-analysis. Conclusions: This work evaluates the evidence of differences in risks between different types of recreational water-based activities in ambient water. The results and conclusions of the systematic review may help decision-makers set activity-dependent recreational water quality standards for the protection of public health. The views expressed here are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

Spatio-temporal dynamics of F-specific RNA bacteriophages in river water during rainfall-runoff events: new data for the water quality risk assessment

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With potential climate change and the increase of extreme rainfall events, water quality and its impact on human health are today important issues. Numerous data in the literature highlighted a higher enteric pathogenic virus contamination in surface water during rainfall events. Nevertheless, the high-frequency monitoring of infectious viral particles during a rainfall-runoff event has never been studied yet. The objectives of this study were: (i) to parameterise the hydrographs to analyse the dynamics and the origins of phage fluxes during rainfall-runoff events and (ii) to provide new data for risk assessment by detection of infectious phage particles during rainfall-runoff events. F-specific RNA bacteriophages (FRNAPH) were used as model to estimate the viral pollution. Two rainfall-runoff events were analysed. One-litre water sample was performed every hour during the course of the studied period. Turbidity was measured for all water samples. Infectious FRNAPH were enumerated by plaque assay (ISO 10705-1) and FRNAPH genogroups were quantified using molecular biology tool (Ogorzaly and Gantzer, 2006). Taking into account the flow of the river (m3/sec), concentrations of FRNAPH were converted in flux (plaque forming units (PFU) or genome copies / sec). An increase of flow rate from 0.5 to 7.1 m3/sec and from 2.6 to 22.5 m3/sec was observed for both rainfall-runoff events respectively. A total of 138 rainfall water samples were collected and analysed. All were positive for infectious FRNAPH. An increase of 2.5 and 1.8 log10 of infectious FRNAPH flux was measured for both events respectively. Genogroups I, II and III were detected. Genogroup II was noted to be the most important in all water samples, with an increase of 1.0 and 1.6 log10 for both events. Same range of variations was observed for genogroups I and III. From a hydrological point of view, a rainfall-runoff event was generally characterised by the relationship between turbidity and water discharge in order to determine sources and transport of sediment. This hysteresis approach was applied to FRNAPH and allowed us to describe the chronological arrival of infectious FRNAPH waves during events. The first arrival of phages, mainly genogroup II, was likely to be linked to the resuspension of riverbed
Aeromonas isolates recovered from the reclaimed water used for irrigation and from the irrigated vegetables at the same time. Objectives: The aim of this study, therefore, was to determine, using molecular tools, the prevalence, diversity and epidemiological relationship of Aeromonas isolates recovered from the reclaimed water used for irrigation and from the irrigated vegetables. Methods: Eleven reclaimed water samples were collected from a wastewater treatment plant, located in Catalonia North-East of Spain. Three water samples were collected after the secondary treatment, three after tertiary treatment that involved chlorination and ultraviolet radiation and five corresponded to irrigation water. The latter came from a hose that extracted water from a well, where the tertiary treated water was accumulated. In addition 3 irrigated vegetables samples i.e.
Oysters and clams collected from the same location exhibit marked differences in Vibrio colonization

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Most human infections from Vibrio vulnificus and V. parahaemolyticus are acquired by eating raw shellfish. Filter-feeding shellfish concentrate these bacteria up to 100-fold over that of the surrounding water. Clams and oysters, both filter-feeding shellfish that are sometimes consumed raw, can often be harvested from the same sites. There has been little research comparing the concentrations of these pathogenic bacteria in different species of shellfish samples collected simultaneously. We collected clams (Mercenaria mercenaria), oysters (Crassostrea virginica), and water samples regularly from various sites along the coast of North Carolina (USA) for over 2 years. These samples were examined for total Vibrio concentrations by plating on TCBS agar. V. vulnificus and V. parahaemolyticus were enumerated by plating samples onto CHROMagar Vibrio, and collecting isolate for subsequent molecular confirmation. We hypothesized that Vibrio loads in clams and oysters would correlate, as both were exposed to the same environmental conditions and exogenous bacterial populations. We found the concentration of total Vibrio, V. vulnificus, and V. parahaemolyticus in water and oysters had a significant linear relationship (p<0.0001, and R-square = 0.33, 0.42, and 0.62, respectively). Clams and water, however, showed no significant linear relationship in total Vibrio concentrations (p=0.62) and
weak relationships with the two Vibrio species (p<0.05, R-square < 0.18, for both). Total Vibrio abundance in clams and oyster also had a weak, but significant, relationship (p=.03, R-square=0.20) that was not seen at the species level. Most interesting was that the concentration of total Vibrio and both V. vulnificus and V. parahaemolyticus was significantly lower (p<0.001, p<0.01, and p<0.05, respectively) in clams than in oysters collected from the same location. This highlights the need for clam-specific environmental research to develop accurate models and broaden ecological understanding. This is especially the case in light of the likely forthcoming US Vibrio requirements for clams.

Disinfection of surfaces in the Ebola context: efficacy assessment of four chlorine types using E. coli and bacteriophage Phi6

Karin Gallandat, Tufts University

Marlene Wolfe; Qais Iqbal; Brittany Mitro; Daniele Lantagne

The 2014 Ebola outbreak in West Africa was the first widespread outbreak and the largest to date. In this outbreak, different - and sometimes contradictory - recommendations were provided by the World Health Organization, the Centers for Disease Control and Prevention, and Médecins sans Frontières on how to disinfect surfaces and clean controlled and uncontrolled spills in Ebola treatment units. The objectives of this research were to: 1) compare the efficacy of four commonly available chlorine solutions (sodium dichloroisocyanurate (NaDCC, pH 6-7), high-test hypochlorite (HTH, pH 9-11), stabilized sodium hypochlorite (NaOCl, pH 9-11) and non-stabilized NaOCl (pH 7)), for the disinfection of three Ebola-relevant surface types; 2) evaluate how recommended practices such as pre-cleaning or covering spills effects surface disinfection efficacy; and 3) determine how presence of a soil load mimicking human liquid waste effects surfaces disinfection efficacy. The test organisms were Escherichia coli (ATCC 25922) and Phi6 (HER #102) propagated in Pseudomonas syringae (HER #1102). The surface carriers were 8-cm discs of stainless steel, heavy duty tarp and nitrile. On each test day, the concentration of each of the four chlorine solutions was confirmed to be within 10% of a target 0.5% solution by iodometric titration. The soil load was prepared according to ASTM International standard and contained 7.80 g/L bovine serum albumin (BSA), 10.92 g/L tryptone and 2.52 g/L bovine mucin. For the evaluation of the surfaces disinfection efficacy, 2 ml of a mixture consisting of 1.36 ml test organism suspension and 0.64 ml soil load or dilution buffer were applied to each surface carrier and left to dry for 1 hour in a biosafety cabinet. Eighteen milliliters of 0.5% chlorine solution were then applied following one of four recommendations (with and without pre-cleaning, with and without covering) and left for 10 minutes. At the end of the exposure time, the discs were placed in a WhirlPak bag containing 300 ml of 0.2% sodium thiosulfate solution and stored on ice. Samples were titrated by membrane filtration for E. coli and by small drop plaque assays for Phi6 within 5 hours of collection. On stainless steel, without pre-cleaning/covering and with pre-cleaning only, no E. coli was detected after exposure to any of the four chlorine solutions and no difference was observed between discs with and without soil load. When the spill without soil load was covered before applying chlorine, no E. coli was detected after exposure to 0.5% NaDCC, HTH and non-stabilized NaOCl. E. coli was detected after exposure to 0.5% stabilized NaOCl; the average reduction was 5.36 log. When pre-cleaning and covering were applied to the spill without soil load, no E. coli was detected after exposure to 0.5% NaDCC and HTH; E. coli was detected after exposure to 0.5% stabilized and non-stabilized NaOCl, with
an average 6.33 and 5.64 log reduction, respectively. When the spill with soil load was covered before applying chlorine, no E. coli was detected after 10 minutes exposure to any of the four solutions. When pre-cleaning and covering were applied to the spill with soil load, no E. coli was detected after 10 minutes exposure to 0.5% NaDCC, stabilized and non-stabilized NaOCl; E. coli was detected after 10 minutes exposure to 0.5% HTH with an average 6.96 log reduction. These results suggest that wiping or covering spills may protect pathogens instead of improving removal. At the time of submission, laboratory work is ongoing. Expected results include the reduction of E. coli and Phi6 on stainless steel, heavy duty tarp and nitrile, with and without soil load, when exposed to each of the four 0.5% chlorine solutions for 10 minutes following four different recommendations for pre-cleaning and wiping. In further steps, we will determine the minimum required exposure time to ensure safe disinfection of surfaces under the tested conditions. Our results should help develop evidence-based recommendations for the disinfection of surfaces in the Ebola context and in emergency situations in general.

Selection of a biosafety level 1 surrogate for the Ebola virus: comparison of bacteriophages MS2, M13, Phi6 and PR772

Karin Gallandat, Tufts University

Daniele Lantagne

The 2014 Ebola outbreak in West Africa was the first widespread outbreak and the largest to date. The outbreak highlighted the need for substantially more research on the Ebola virus (EV), but this was limited by the relatively few laboratories capable and certified to work with the BSL4 Ebola virus. Bacteriophages are commonly used as surrogates for viral organisms, however, it is not known which bacteriophages are an appropriate surrogate for EV. The goal of this research was to determine which of four bacteriophages would be the most appropriate surrogate for EV when testing the efficacy of disinfecting surfaces and hands with chlorine compounds. The test organisms were: MS2 (ATCC 15597-B1) and M13 (ATCC 15669-B1), both using Escherichia coli (ATCC 15597) as a host; PR772 (HER #221) propagated in Escherichia coli (HER #1221); and Phi6 (HER #102) propagated in Pseudomonas syringae (HER #1102). The inactivation mechanisms involved in chlorine disinfection are still poorly understood and have alternatively been attributed to genome, protein and envelope damage. MS2 is an ssRNA virus like EV, M13 is filamentous, Phi6 is enveloped and PR772 is a lipid-containing bacteriophage-chosen as an alternative to a second enveloped phage. All four bacteriophages were propagated with the double agar overlay method and recovered by diffusion; the resulting suspension was filtered at 0.22 µm. The experimental setup described by Cook et al. (2015, Viruses, vol. 7), using actual EV, was replicated as closely as possible for each bacteriophage. Solutions of 0.1% and 0.5% sodium hypochlorite (NaOCl) were prepared with hard water (0.04% CaCO3) and the chlorine concentration was confirmed by iodometric titration. A soil load containing 7.80 mg/ml bovine serum albumin, 2.52 mg/ml bovine mucin and 10.92 mg/ml tryptone was prepared according to ASTM International Quantitative Carrier Testing standard and mixed with the bacteriophage stock (ratio 1:1.47). Surface carriers were 1-cm in diameter type 430 brushed stainless steel discs, sterilized by autoclaving. Ten microliters of the phage/soil load mixture was applied onto each disc and left to dry in a biosafety cabinet. After one hour, 50 µl of a 0.1% or 0.5% NaOCl solution was applied for 1, 5 or 10 minutes. At the end of the exposure time, 950 µl of 0.25% sodium thiosulfate solution were added to neutralize the
chlorine and the samples were recovered for titration. All tests were performed in triplicate. For EV, Cook et al. (2015) reported 0.7 and 2.8 log reductions after 1 and 10 minutes exposure to 0.1% NaOCl; and 2.2 and >6.6 log reductions after 1 and 10 minutes exposure to 0.5% NaOCl. In our experiment, M13 was reduced by 1.27 and 3.01 log after 1 and 10 minutes exposure to 0.1% NaOCl; and by 3.07 and 3.57 log after 1 and 10 minutes exposure to 0.5% NaOCl. PR772 was reduced by 1.62 and 4.10 log after 1 and 10 minutes exposure to 0.1% NaOCl; and by >5.80 log after 1 minute exposure to 0.5% NaOCl. Phi6 was reduced by 1.85 and 3.50 log after 1 and 10 minutes exposure to 0.1% NaOCl, and by 1.79 and >5.70 log after 1 and 10 minutes exposure to 0.5% NaOCl. Preliminary results for MS2 suggest that it is reduced by 1.70 log after 10 minutes exposure to 0.1% NaOCl and by 3.00 log after 10 minutes exposure to 0.5% NaOCl. We found MS2 and M13 are more resistant than EV and thus would yield overly conservative results if used as surrogates. Additionally, we found PR772 was an inappropriate surrogate as it was too easily inactivated. Phi6 exhibited an intermediate behavior and is slightly more resistant than EV, which is ideal for a surrogate organism. We therefore recommend using Phi6 as a surrogate for evaluating the efficacy of chlorine disinfection against EV. Our results suggest that the envelope might play a key role in determining the resistance of viral organisms to chlorine but further and more fundamental research is needed to understand inactivation mechanisms.

**Characterization of the microbiological quality of two non-potable water reuse systems in Minnesota**

Cheryl Haines, University of Minnesota - Twin Cities

Mark Borchardt; Satoshi Ishii; Timothy LaPara

Characterization of the microbiological quality of two non-potable water reuse systems in Minnesota.

Cheryl Haines, Mark A. Borchardt, Satoshi Ishii, and Timothy M. LaPara Many cities and water managers in Minnesota are implementing new stormwater and rainwater use systems to reduce the demand for high quality potable water and to better manage stormwater. However, questions remain regarding the impact these systems may have on public health due to the elevated presence of pathogens in stormwater and rainwater. In order to examine these risks, two water reuse systems in Minnesota were examined: a system in which rooftop rainwater was collected and used for toilet flushing and a system in which water from a stormwater collection pond was used to irrigate athletic fields. The bacterial content of each system was investigated using both cultured-based techniques and microfluidic quantitative PCR. Culture-based techniques were used to investigate the presence of total coliforms, Enterococcus spp. and E. coli, while qPCR was used to target all bacteria (16S rRNA genes), Legionella spp., and numerous other bacterial pathogens such as Campylobacter jejuni, E. coli O157:H7 and Salmonella typhimurium. Rainwater used for toilet flushing had significant quantities of culturable fecal indicator bacteria (FIB) when compared to tap water, which suggests the rainwater was of lower quality. Total bacterial levels (16S rRNA genes) fell within expected values for the sample types, while Legionella pneumophila was present at elevated levels when compared to tap water. In addition, the impact of ozonation/disinfection was shown in the rainwater system, as storage cisterns showed higher levels of FIB, Legionella spp., and total bacteria (16S rRNA genes) than rainwater collected from the toilet. Stormwater used for the irrigation of athletic fields also had significant quantities of culturable FIB when compared to groundwater (negative control), which suggests this stormwater is of lower quality than the groundwater found in the area. Quantities of all bacteria (16S rRNA genes) fell within expected values, while Legionella spp. were present at elevated levels when compared to
controls. In addition, the impact of depth within the stormwater pond was noted; stormwater collected from a depth of 8 feet contained higher levels of all bacteria (16S rRNA genes) and Legionella spp. than water collected from just below the water surface. These results demonstrate that significant quantities of FIB are found in rainwater used for toilet flushing and stormwater used for the irrigation of an athletic field, and that both rainwater and stormwater were of lower quality than tap or groundwater, which suggests there could be an elevated risk to public health associated with these creative uses of non-potable water.

**Rapid Detection of E. coli in 8 hr from Beach Water Using KwikCount EC Medium**

Fu Chih Hsu, Scientific Methods Inc

Rebecca Wong

To protect public health in beach, a rapid test to determine concentrations of E. coli is needed. A new medium, KwikCount EC, is developed and evaluated for enumerating E. coli in 8 hr from 10 beach water samples. This medium contains a fluorogenic/chromogenic enzyme substrate mixture, a special mixture of nutrients to promote the growth of E. coli, and an inhibitor to suppress growth of non-target bacteria. By incubation at 41°C, blue fluorescent colonies (E. coli) can early be observed within 8 hrs under UV light. This method makes it possible to achieve the same day monitoring of beach water microbiological quality. EPA Alternate Test Procedure (ATP) was adopted to evaluate KwikCount EC medium and compare with the reference method, EPA 1603 using modified mTEC agar, to analyze 10 fresh beach water samples. The mean recovery, precision, and false-positive rate will be obtained and compared to the reference method, modified mTEC agar. Based on the statistical analysis, there was no significant difference in recoveries of E. coli among 10 sources of fresh beach water although KwikCount EC has slightly higher recovery (78.7% vs. 76.6%). For individual source water, there was no significant difference in 7 sources beach water samples. KwikCount EC recovered more E. coli than modified mTEC in 2 sources water samples (Potato Creek and White Rock Lake) and less in one source water sample (Chatfield). When comparing precision for both methods, precision by KwikCount EC was slightly better than modified mTEC (22.0% vs. 24.7%), but no significant difference was found by statistical analysis. The false positive rate and false negative rate by KwikCount EC was 10.4% and 1.4%, respectively. The higher false positive identification by colony verification procedures in typical colonies may due to run-off of liquid medium (KwikCount EC broth) and mixed different strains of bacteria in individual colony on the membrane. The overall false negative rate from 10 sources of fresh beach water is was very low (1.4%). Based on these results, it is feasible to detect E. coli from beach water in 8 hr by KwikCount EC medium. There is no statistic significant difference in recoveries and precisions between the proposed method (KwikCount EC) and the reference method (modified mTEC). It can be concluded that performance by these two methods is equal.

**Evaluation of PMA-qPCR for Quantitative Differentiation of Live Human-associated Bacteroidales for Water Quality Monitoring**

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Quantitative Polymerase Chain Reaction (qPCR) is a rapid and sensitive technique for detecting potential sources of fecal pollution in ambient surface waters. Propidium Monoazide (PMA) treatment has been identified as a method to distinguish between live and dead cells in qPCR analyses for fecal source tracking. PMA-qPCR can also potentially help distinguish between recent and past fecal contamination. This study evaluates the suitability of PMA treatment using the human associated Bacteroides HF183 qPCR marker for the effective discrimination of live and dead cells in ambient water samples. The methodology was standardized with a B.dorei pure culture at the Florida Department of Environmental Protection laboratory. Various PMA concentrations and light exposure times were tested to determine the optimal conditions for the PMA-qPCR method. Three light boxes using high intensity blue LED lights (460 nm wavelength) were built in-house to achieve simultaneous exposure of up to 7 sample filters. Since Bacteroides are obligate anaerobes, spiked sewage samples were tested at four time intervals (up to 48 hours) to evaluate the persistence of viable cells after exposure to an aerobic environment. After standardization, the method was tested with untreated and treated sewage from a local wastewater facility and also with surface water samples from water bodies that have shown consistently high fecal coliform results using standard microbiological culture methods. Quantification was based on the Ct difference between qPCR and PMA-qPCR analyses to determine the percent viable cells and total cells in a sample. Results indicate PMA-qPCR is an effective method to distinguish live from dead cells using the human-associated Bacteroidales marker. Preliminary results with wastewater influent and environmental water samples further suggest that a high percentage of viable cells corresponds to the presence of recent (up to 48 hours) fecal contamination from untreated sewage and a low percentage of viable cells can indicate a source of past contamination or of treated sewage.

Metagenomic characterization of Vibrio in the Neuse River Estuary, NC

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Brett Froelich; Rachel Noble

The genus Vibrio encompasses a diverse and abundant group of heterotrophic bacteria which are ubiquitous and abundant members of the native flora in coastal and estuarine waters. Though most are benign commensals, several Vibrio species are important human and animal pathogens. These organisms are common in marine waters and shellfish and proliferate rapidly in warm, moderately saline waters. However, their responses to other environmental parameters (nutrients, chlorophyll-a, turbidity, etc.) are not well understood, and traditional molecular and culture-based analyses have failed to resolve these relationships. In order to better understand Vibrio responses to changing environmental gradients, we have conducted whole-metagenome shotgun sequencing and multivariate analyses for samples collected in the Neuse River Estuary (NRE). This approach has allowed us to identify previously uncharacterized relationships between Vibrio abundance and common water quality parameters, including dissolved organic carbon and dissolved organic nitrogen. This study is among the first to use next-generation sequencing technologies for the ecological characterization of Vibrio species, and will be followed by a year-long spatial and temporal 16S metagenomic survey in the NRE in order to further characterize the relationship between Vibrio and bacteria in the NRE.
the environment.

Health Risks Associated with Integrated Aquaculture in Rural China: Focusing on Zoonotic Pathogen and Antibiotic Resistance Hazards

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Gary Klase; Seungjun Lee; Song Liang

Background: Aquaculture is a major economic activity in many countries, especially developing countries. Integrated aquaculture operations, those that utilize animal waste or plant residue as a fertilizer for fishponds, can be a resource-efficient method of producing food for consumption or sale. However, the use of human or animal excrement as a pond fertilizer carries certain risks to consumers and workers that should be understood and addressed. Objectives: The purpose of this study was to investigate microbial water quality and potential health hazards associated with rural aquaculture operations in the Jiangmen city region, Guangdong province, China. The area was chosen since it is also known as an endemic region of Clonorchis sinensis, which is a liver parasite. Approach: Twenty five fishponds across ten villages were sampled between June and August of 2012. The degree of fecal contamination (E. coli) in each pond was measured and microbial source tracking (MST) methods were used to identify ponds which were contaminated with human feces and/or pig feces. Antibiotic resistance (tetQ and sul1) was also investigated together with pathogens (Salmonella, Arcobacter, Shiga toxin-producing E. coli, microcystin-producing Microcystis) and water quality parameters. Nine ponds (from the areas of liver fluke prevalence) were also examine for their microbial community structure using next generation sequencing. Major findings: The results show high concentrations of fecal contamination in aquaculture ponds (94% of the ponds exceeded the US EPA criteria for recreational water) and MST confirms that, in addition to pig waste produced during animal production, many of the ponds are also contaminated with human fecal matter (84%). Many of these ponds also tested positive for antibiotic resistant genes (88% of the ponds, positive for tetQ and 72% for sul1). Salmonella was the major pathogen detected (50% of the ponds) and the DNA sequencing shows that S. Typhimurium, S. Shubra, and S, Infantis were the major species. The microbial community structure shows that the ponds with human/animal fecal contamination harbors an array of human pathogens. Conclusion: These findings suggest much elevated health risks to workers and community members who come into contact with the water from these fishponds, as well as a potential risk to consumers of fish from these ponds. The domestic animals, especially pig, may play a role in the epidemiology of zoonotic disease in the region.

Pathogen Indicator Reduction and Bacteria Antibiotic-Resistance Evaluation in Dairy Manure Separation Using Polymer

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Water problems are largely related to pollutions from agricultural related practice. In Wisconsin, run-
off from dairy farms and other agricultural activities has seeped into aquifers and elevated levels of Nutrients. However, the emphases of most environmental policies and studies concerning manure management have been on the effects of nutrient recovery and water quality. Nevertheless, the microbial quality of manure should not be neglected since many outbreaks of gastroenteritis related to livestock operations have been reported. In this paper, evaluation and reduction of antibiotic-resistance bacteria in dairy manure are emphasized because the abundance of antibiotic resistance bacteria in agricultural soils, ground water, and surface water may be enhanced by large dairy farm management practices followed by land application of dairy manure which has not been treated properly. Appropriate manure treatments are needed to reduce the potential risk of exporting antibiotic resistant bacteria to environment and also reduce antibiotic resistant bacteria exposure to animals if processed water is recycled. Results from this research revealed antibiotic (cephalosporin, florfenicol, penicillin, and tetracycline) resistance bacteria present in manure from a large dairy farm in Wisconsin. Manure separation under relatively low speed centrifuge with 100ppm polyacrylamide (PAM) emulsion addition reduced bacteria indicators population such as total coliforms and E. coli significantly in the liquid stream compared to no PAM added. However, the percentages of antibiotic resistant isolates in liquid stream after centrifuge e with PAM were higher compared to raw manure and no PAM added. The antibiotic resistant bacteria isolates are then identified and documented using 16S rRNA sequencing.

**Propagation and purification of Bacillus sp. endospores for use**

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Spores produced by some bacteria in response to unfavorable conditions are not inert particles but rather cells in a latent physiological state. Often found in soil and water, spores are highly resistant to toxic compounds, temperature, desiccation and radiation. These properties and the ability to germinate selectively in response to environmental triggers, make them ideal candidates for biotechnological and environmental applications. Aerobic spores of Bacillus subtilis, are increasingly being used as a surrogate in bio-colloid transport studies at laboratory and field-scales, both in water treatment plants and as a conservative indicator of Cryptosporidium parvum oocysts transport in the subsurface. There are currently no standardized preparation methods for spores used in such demonstrations. Variability in preparation methods (i.e. propagation, purification and storage) can affect surface properties and the production of extracellular polymeric substances (EPS) which in turn may affect attachment/detachment of spores and other particles in transport studies. Appropriate assessment of pathogen surrogate passage through filtration processes is a critical component of quantitative microbial risk assessment; sources of uncertainty (such as preparation protocol) should be addressed and minimized whenever possible. Herein three strains of Bacillus sp. (two laboratory strains and one environmental isolate) are studied using various bacteriological media and preparation protocols to evaluate changes in surface characteristics (i.e. morphology, size, zeta-potential, hydrophobicity) and EPS production. Recommendations for standardization of spore preparation procedures, aiming at maximizing yields and minimizing change in surface properties in order to decrease uncertainty and improve experimental comparability, are presented.
Taking Evidence-Based Microbiology to the Community Level in Lower Nyakach, Kenya, to Eliminate Waterborne Diseases

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Dinah Chienjo

MOTIVATION: WHO estimates that waterborne diseases among a billion people living in extreme poverty EVERY DAY leads to >4,600,000 people suffering from diarrhea and >2,000 deaths. Most of these people must use contaminated unimproved drinking water sources and have no expectation that they will be connected to a safe water supply in the foreseeable future. We have developed and implemented a successful and replicable strategy with the goal of eliminating waterborne diseases in the Friends of the Old (FOTO) project in Lower Nyakach, Kisumu County, Kenya, where 70,000 people live without electricity and plumbing and must obtain their drinking water from heavily contaminated rivers, streams, ponds and shallow wells. METHODS: The first step was for the FOTO staff to take evidence-based microbiology to communities to demystify microbiology and have everyone understand the relationship between contaminated drinking water and disease. Two tests for Escherichia coli from the water and food industries were used: the Colilert® 10 ml presence/absence test (IDEXX, Westbrook, ME) and the E. coli/Coliform Count PetrifilmTM (3M, St. Paul, MN), a quantitative test for 1 ml. These tests were inoculated directly, incubated overnight, and provided easily interpreted results the next day that correlated with WHO disease risk categories: low, moderate, high, or very high. After testing and teaching made communities aware their drinking water sources had a high/very high disease risk, all 14,000 households were given a bottle of commercially available 1.2% sodium hypochlorite every other month, and all 79 schools received chlorine during the academic year. FOTO staff went door to door to make sure households used chlorine correctly all the time. In addition, 1000 households have been given a simple solar cooker that uses sunshine to pasteurize water by heating to 65°C, which is verified by the wax melting in a reusable Water Pasteurization Indicator. RESULTS. Village elders report that the FOTO effort has resulted in 90% of households always treating water with chlorine. A group that doesn't approve of chemicals always heats their drinking water. From February-May, 2015, cholera outbreaks in two districts adjacent to Lower Nyakach resulted in 1292 cases of cholera and 17 deaths. There were no cholera cases in Lower Nyakach. CONCLUSION: Evidence-based microbiology, community education, and door to door visits by people they know and trust resulted in a project that has nearly eliminated waterborne diseases that plagued Lower Nyakach before the FOTO project. The simplicity and effectiveness of the FOTO project could be easily replicated worldwide to aim for the elimination of waterborne diseases among those in extreme poverty.

Treatment Options for Antibiotic Resistance Genes in Wastewater

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Amy Prudent

Wastewater treatment plants can potentially serve as control points to reduce dissemination of biological contaminants, such as antibiotic resistant bacteria (ARBs) and antibiotic resistance genes
(ARGs), to the environment in treated effluent and finished biosolids. Anaerobic digestion is commonly employed by many utilities to meet USEPA Part 503 regulations pertaining to the biosolids treatment for the purposes of land application or beneficial reuse. Optimizing or adapting digestion processes to reduce or remove ARBs and ARGs from land-applied biosolids could benefit the global war on antibiotic resistance through limiting the dissemination, transfer, emergence, and infiltration of novel antibiotic resistance vectors from environmental reservoirs into clinical settings. Applying a mass balance approach to anaerobic digestion reveals two variables that potentially impact ARG content of treated biosolids: ARG concentration in influent sludge and ARG transformations within the digestion process. ARG fate can be linked to the fate of the ARB housing the gene. As such, ARGs enter a digester via association with an ARB host or presence of extracellular ARG DNA in the sludge matrix. Transformations in the digester could include ARB death, replacement growth, or selective enrichment; ARB ejection of a plasmid containing ARG(s); horizontal gene transfer (HGT) of ARGs; or chemical or biological degradation of extracellular ARG DNA. This paper summarizes our work towards characterizing these variables. Key conclusions from our work include: ? ARG fate is largely driven by ARB survival or death, but gene transfer also plays a role. ? HGT is important in both mesophilic and thermophilic digestion and biosolids storage for ARGs associated with Class 1 integrons. ? HGT was stimulated by cold temperature storage of treated biosolids, perhaps as a stress-induced response. ? No HGT or selection pressure enrichment was observed when thermophilic digesters were exposed to antimicrobials in lab-scale, semi-continuously fed digester studies. In all, thermophilic digestion may provide greater reductions in ARGs than mesophilic digestion because the microbial diversity is more limited, which may reduce raw sludge ARB survival and reduce opportunities or compatibility for HGT.

**Delineating the microbial ecology of phage-prokaryotes in hypersaline environment through "omics" approach**

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Majority of metabolic processes related to nutrient, carbon, and metal cycling in the ecosystems' sediments are prokaryotic mediated and their diversity provides environmental sustainability to the ecosystem. On the other hand, bacteriophages as the most abundant biological entities on the planet play a significant role in microbial population dynamics within an ecosystem. Although bacteriophages do not directly participate in any metabolic activity, but through their infection cycles they directly affecting the prokaryotic community and resiliency of the ecosystem. Although the diversity of bacteriophages in the natural ecosystems in general has been studied, but still not much is known about the bacteriophage diversity and their role in bacterial communities population in hypersaline environment such as Great Salt Lake, Utah. In this research, we investigated the prokaryotes and bacteriophages diversity, population, and their role in various nutrient cycles in the Great Salt Lake using metagenomics analysis. Following collection of sediment samples from deep brine layer of Great Salt Lake, bacteriophages were extracted and purified using CsCl gradient and DNase treatment. Nucleic acids of bacteria and phages were extracted using PowerMax Soil DNA isolation kit and Norgen phage DNA isolation kit, respectively. In order to minimize any biases in our analysis, no amplification processes was carried out on the phage DNA sample. Bacterial and phage DNA samples were sequenced on Illumina MiSeq DNA sequencer with 300-cycle paired-end at HCI Core Facility, University
of Utah. Using CLC Genomics Workbench, raw reads were quality filtered followed by scaffold assembly with 500 bps as minimum length for the contigs. To understand the prokaryotes' and phages' diversity, taxonomic binning of the contigs was performed using tBLASTx against NCBI non-redundant nucleotide and RefSeq viral database, respectively followed by LCA analysis using MEGAN v.5 to generate taxonomic cladogram. For gene analysis and possible gene transfer between phage and their host, gene prediction of the prokaryote and phage contigs were performed using MetaGeneMark v.2.8 followed by COG analysis for gene annotation. The prokaryote taxonomic rRNA analysis revealed more than 450 different genera classified in the sediment sample while most abundant genera of the annotated contigs were belong to taxa involved in various biogeochemical cycles. Among them, proteobacteria (46.2%), firmicutes (20.3%), bacteroidetes (9%), actinobacteria (5.3%), chloroflexi (5.2%), cyanobacteria (2.7%), and planctomycetes (1.7%) were the most dominant bacterial phyla present in the sediment sample. On the other hand, a high resolution insight into the viral biogeography was developed by comparing the abundance of the novel unclassified Caudovirales isolated from Great Salt Lake sediments with other phage metagenomes obtained from freshwater lakes, lake sediments, and hypersaline marine environments. In addition, gene prediction in the bacterial and phage contigs showed the shared functional genes in the prokaryote and phage samples, which could have originated from gene transfer process between the bacterial hosts and phages. Our in-depth metagenomic analysis allowed us to comprehensively characterize interactions between phage and prokaryotic communities of the Great Salt Lake and determine how these interactions affect the diversity and function of these communities and their contributions to biogeochemical cycles.

An Integrated Monitoring Approach to RTCR Level 1 and 2 Assessments

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When evaluated in 2010, the EPA estimated that 10.5 million people in the United States rely on potable ground water from small water systems known as transient non-community water systems (TNCWS). These systems typically serve tens to hundreds of people at a time and are often located in rural areas which are susceptible to contamination from human activities including septic systems and animal agriculture. While many TNCWS are not required to provide any level of treatment under the Safe Drinking Water Act, current drinking water regulations require monitoring of total coliforms under the Total Coliform Rule. However, by April 1, 2016, systems must begin to comply with the Revised Total Coliform Rule (RTCR) which places additional burden on TNCWS with unsafe monitoring samples. One unsafe sample can lead to costly and time consuming follow up testing and increased future routine monitoring. In addition, the RTCR introduces Level 1 and Level 2 assessments which require either the public water supply owner or the state government to assess potential sanitary defects in the system that may have led to an unsafe sample. In Wisconsin, the Department of Natural Resources (WDNR) has selected to conduct both levels of assessment because many TNCWS owners lack the technical training or scientific background to provide a comprehensive assessment that optimizes protection of public health and safety. The time and cost requirements associated with these assessments and increased system monitoring if a cause and remedy is not found is significantly magnified in Wisconsin, which is home to roughly 10% of the nation’s TNCWS (approx. 9400 active systems, DeWeese, WDNR, pers. comm., 2016). Thus, the objective of this research project is to
develop a scientifically based well assessment protocol to be used by the WDNR to address the monitoring and assessment changes under the RTCR. The program focuses on integrating large volume sampling (100L of well water), hollow fiber ultra-filtration concentration of that sample, measurement of microbial indicators, ATP measurements, enterobacteria identification, molecular testing (qPCR) for fecal source tracking (FST) targets, and land use information to accurately, cost-effectively, and quickly assess microbiological contamination in TNCWS. All analytical results are then used to inform corrective action. The scientific information provided to the property owners has catalyzed significant buy-in for costly remedies when needed.

**EPA's Development of Recreational Water Quality Criteria for Coliphage: Updates and Coliphage Experts Workshop Overview**

Sharon Nappier, US Environmental Protection Agency

EPA provides Recreational Water Quality Criteria (RWQC) to protect the designated use of primary contact recreation. EPA recommends adoption of RWQC into state water quality standards to develop point source permits, assess waters, and provide beach notifications. Historically, EPA's RWQC recommendations have been based on the fecal indicator bacteria E. coli and enterococci. EPA is now evaluating coliphage, a viral indicator, to better help prevent viral associated illnesses. EPA recently held an Experts Workshop to engage internationally recognized experts on the state of the science of coliphage and their usefulness as a viral indicator for the protection of public health. Topics for discussion included: the need for a viral indicator; coliphage as a predictor of gastrointestinal illnesses; coliphage as an indicator of wastewater treatment performance; male-specific vs somatic coliphage; systematic literature review of viral densities; and future research. EPA will provide an overview of the recent Experts Coliphage Workshop and an update on the development of RWQC for coliphage.

**Evaluation of microbiological risks associated with direct potable reuse**

Sharon Nappier, Environmental Protection Agency

Jeffrey Soller; Sorina Eftim; Isaac Warren

Background: Water reuse has become an increasingly important water supply option in many regions of the world facing water shortages. The United States currently lacks national regulatory recommendations or criteria values for recycled water, although states and municipalities have begun regulating or permitting indirect potable reuse (IPR) and direct potable reuse (DPR) facilities. Due to nature of the source water (raw influent), there is a need to quantitatively evaluate the health risks associated with exposure to microbial contaminants from the various multi-barrier treatment strategies under consideration. Given levels of pathogens in raw sewage and target levels in product water, monitoring product water from advanced water treatment facilities (AWTF) to determine whether adequate treatment is occurring is technically challenging. To our knowledge, this is the first use of quantitative microbial risk assessment (QMRA) methods to evaluate various multi-barrier DPR treatment strategies. Objectives: Our objectives were to provide a literature review of ranges of reference pathogens in raw sewage and of their removal in various individual unit treatment
processes, and to conduct a microbial risk assessment to understand the potential public health implications of various DPR options. Overall this work provides an illustration of a QMRA tool that can be used by managers to assess risk associated with a proposed DPR treatment project. Methods: Peer-reviewed literature was used to characterize the density of each reference pathogen in raw wastewater and the reduction of each reference pathogen across individual unit treatment processes. Using this literature review, a stochastic static QMRA methodology was used to estimate infection from multiple pathogenic microorganisms through ingestion of DPR product water from the four representative AWTF treatment train configurations. A two-level Monte Carlo numerical simulation of randomly selected influent and attenuation values was used to estimate pathogen-specific daily risks in the DPR product water. Cumulative daily and annual risks were then computed for each treatment train. A sensitivity analysis was conducted to further evaluate the impact of selected dose-response models for two reference pathogens. Results and Conclusions: The results of this evaluation illustrate quantitative health advantages for DPR projects circulating their product water through a conventional drinking water treatment facility, compared to DPR projects introducing their product water directly into the potable water distribution system. Additionally, annual risk estimates for any given DPR treatment train are typically driven by the highest daily risks for any of the reference pathogens. The analysis also indicates that norovirus is a particularly important reference pathogen that should be carefully considered in future DPR projects. Finally, the QMRA methodology is adaptable to other DPR treatment trains and could be iteratively refined as additional data become available. Overall, this work will be useful for federal and state regulators considering DPR as source water, state and local decision makers as they consider whether to permit a particular DPR project, and design engineers as they consider which unit treatment processes should be employed for particular DPR projects.

Systematic literature reviews and development of distribution curves for viral densities in raw wastewater

Sharon Nappier, Environmental Protection Agency
Sorina Eftim; Tao Hong; Audrey Ichida; Isaac Warrend; Jeffrey Soller

The United States Environmental Protection Agency (EPA) is developing Recreational Water Quality Criteria for coliphage, a viral indicator, to ensure public health protection from water sources that have been influenced by fecal contamination. EPA is considering use of a quantitative microbial risk based approach to support the criterion development. The methodology relies on densities of key viral pathogens and coliphages in wastewater influent (raw sewage). EPA conducted a systematic literature review of published peer-reviewed publications to identify norovirus, adenovirus, and coliphage density data in wastewater influent. Pathogen-specific study inclusion criteria including scope, study quality, and data availability were applied to each publication. A non-parametric bootstrap statistical model was used to estimate the distribution of the aforementioned viruses. This statistical approach accounts for heterogeneity in study-specific distribution curves, sampling locations, and sampling seasons and provides a comprehensive representation of the data. Initial results suggest noroviruses in raw wastewater are well represented by distributions with means in the order of 104 - 106 genome copies/L, somatic coliphages in raw wastewater are well represented by distributions with means in the order of 105 - 107 pfu/L, and male-specific coliphages in raw wastewater are well represented by distributions with means in the order of 105 - 106 pfu/L. Methods presented are reproducible and can
be used to evaluate densities of other viral pathogens in wastewater influent, effluent, and ambient waters.

**Impact of environmental fecal contamination on hand hygiene in urban Harare**

Tala Navab-Daneshmand, Eawag

Max Friedrich; Linn Mlambo; Tamuka Nhwatiwa; Hans-Joachim Mosler; Timothy Julian

In this study we investigated the impact of environmental fecal contamination on hand hygiene and handwashing effectiveness. Diarrheal disease is responsible for 9% of all deaths in children under five worldwide. Enterotoxigenic and enteropathogenic Escherichia coli have been identified amongst the pathogens most responsible for moderate to severe diarrhea. A microbial survey of E. coli in environmental reservoirs (hands, drinking water, handwashing water, and soil) and fecal samples (humans and chickens) was conducted in urban households (n = 147) of Harare, Zimbabwe. A subset of samples ? drinking water (n = 50), handwashing water (n = 50), soil (n = 50), human feces (n = 55), and chicken feces (n = 35) ? was also analyzed for E. coli O157:H7, a serotype that is the most common enterohemorrhagic E. coli in relation to public health. Overall, E. coli was detected in 42 to 68% of environmental samples (drinking water, handwashing water, and soil); and 85% of hand rinse samples before handwashing. E. coli concentrations on hands ranged form < 0.5 to 3.9 log10 CFU per hand. E. coli concentrations in drinking water, handwashing water and soil samples ranged from < 0 to 3.3 log10 CFU per 100 mL, < 0 to 3.4 log10 CFU per 100 mL, and < -0.9 to 1.4 log10 CFU per g total solids, respectively. Although 49% of human feces and 97% of chicken feces contained E. coli O157:H7, this serotype was only detected in 2% of drinking water and 8% of handwashing water and soil samples. A logit model showed E. coli contamination was not a predictor of presence/absence of E. coli O157:H7 for drinking water (p = 0.36), handwashing water (p = 0.26) and soil (p = 0.22). Moreover, E. coli contamination on hands after handwashing was correlated with the E. coli levels in the handwashing water (Spearman’s rho 0.21, p = 0.09). This study identifies an important relationship between household hygiene and hand hygiene in Harare, Zimbabwe.

**Inactivation and Growth of Bacterial Indicators and Pathogens in Greywater in the context of Blue Diversion Autarky Toilet**

Mi Nguyen, EAWAG

Allemann Lukas; Ziemba Chris; Larive Odile; Morgenroth Eberhard; Julian Tim

The Blue Diversion Autarky Toilet (BDT, www.autarky.ch) was developed to provide a safe and affordable sanitation technology for people who lack access. The BDT provides flushing, hand washing, and personal hygiene water which, after use, is collected for treatment and then for recycle. Greywater treatment in the BDT includes a biologically activated membrane bioreactor (BAMBi) followed by a post treatment technology. To identify an effective post treatment, granular activated carbon (GAC), ultraviolet C (UVC), chlorine, and electrolysis were tested and benchmarked based on their performance in inactivating and reducing growth of bacteria. Two indicators (Escherichia coli and
Enterococcus spp.) and two pathogens (Pseudomonas aeruginosa and Salmonella typhimurium) were chosen to study behaviors of waterborne pathogens in greywater. Bacterial concentrations were measured using culture-based method (spreading on selective-media plates) and flow cytometry (total cell counts and intact cell counts). E. coli, P. aeruginosa, and S. typhimurium demonstrated potential for limited growth in water following treatment with the BAMBi. Adding a carbon (E. coli and P. aeruginosa) or iron (S. typhimurium) source increased growth potential (final cell concentration in stationary phase), suggesting carbon or iron limitation. The addition of a 6 L-GAC column after BAMBi contributed up to 94% removal of dissolved organic carbon (DOC) and 96% reduction of assimilable organic carbon (AOC). The GAC also reduced growth potential for E. coli by 2 log10 (99%). Culture-based method and flow cytometry showed good agreement on monitoring the growth of bacteria in BDT waters. In water after BAMBi+GAC, all three post-treatment options (chlorination, electrolysis, and UVC irradiation) achieved good inactivation of E. coli and P. aeruginosa. Both E. coli and P. aeruginosa were reduced more than 5 log10 of during direct chlorination (sodium hypochlorite; from 0.5, 1.7, and 3.4 mg Cl2/L) after 5 min of exposure. However, reactivation was observed for both bacteria in water that was treated with chlorine at low concentrations and then quenched by sodium thiosulfate (≤0.5 mg Cl2/L for E. coli and ≤1.7 mg Cl2/L for P. aeruginosa). Similarly, both bacteria were effectively inactivated (5 log10 for E. coli and 4 log10 for P. aeruginosa) within 30 min of exposure to UVC (JBL, 5 W, 9μW/cm2), but reactivated after 72 h in the dark. Electrolysis (Condias; 0.5, 5, and 20 W) showed good inactivation of both bacteria (5 log10 inactivation after 5 min) and no reactivation in the presence of chlorine quencher (sodium thiosulfate). Treatment including the BAMBi, GAC, and electrolysis appear to be promising technologies for inactivating and reducing reactivation or growth of bacteria for greywater reuse.

**Campylobacter species, Salmonella serotypes and application of RNA approach for microbial source tracking at the River Kokemäenjoki watershed**

Tarja Pitkanen, National Institute for Health and Welfare

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Accurate identification of the pathogen species and the fecal pollutant sources is of great importance in waterborne health risk assessments and in safeguarding the microbial water quality of watersheds. The infectivity rates between the different zoonotic pathogens originating from different pollution sources may vary greatly. Therefore the genus level information about the presence of pathogens alone is not sufficient for risk assessments. Furthermore, the identification of fecal pollution sources using DNA-based quantitative PCR (qPCR) methods do not provide viability information of the target bacteria since the DNA of dead microbes may persist in the environment. In this study, we identified the Campylobacter species and Salmonella serotypes isolated from the River Kokemäenjoki watershed in Finland and applied RNA-based RT-qPCR techniques to characterize active microbial populations in these waters. Surface water, groundwater and sewage effluent sampling was conducted quarterly in 2012-2014 at a total of 32 locations. Seventy-six sewage effluent, 119 surface water and 38 treated water samples from Kokemäenjoki watershed were collected. The counts of E. coli and intestinal enterococci were enumerated using membrane filtration methods with media CCA with antibiotics and S&B, respectively. The semi-quantitative count estimates of thermodetolerant Campylobacter spp. were obtained using in selective Bolton and Preston enrichment broths prior plating on mCCDA according to
ISO 17995 method. Campylobacter species distribution was investigated with PCR-REA and MALDI-TOF methods. For isolation of Salmonella spp., ISO 19250 method with buffered peptone water resuscitation and RV enrichment was used together with MSRV medium prior plating on XLD and Brilliance agar media and the selected isolates were serotyped. Human, ruminant, swine and gull-specific molecular markers (HF183, Rum-2-Bac, Pig-2-Bac and Gull4) as well as Bacteroidetes spp. (GenBac3) were quantified using rRNA-targeted RT-qPCR and rDNA-targeted qPCR assays. Fecal indicator bacteria were detected in all studied surface water samples, the mean counts were 27±54 CFU 100ml-1 and 28±50 CFU 100ml-1 for E. coli and enterococci, respectively. Thermotolerant Campylobacter spp. were detected in 84% of surface water samples (100 out of 119) with medium count of 5 CFU L-1 (range 0.5-500 CFU L-1). Campylobacter jejuni, Campylobacter coli and Campylobacter lari were detected in 70, 10 and 54 samples out of 100 Campylobacter spp. positive samples. Salmonella enterica was isolated from 20 out of 119 surface water samples (17%) with medium count of 0.3 CFU L-1 (range 0.3-9.1 CFU L-1). S. enterica serovar Typhimurium was the most common isolate followed by Enteritidis, Apeyeme, Bredeney, Newport and Panama. All surface water samples in the studied watershed contained human-specific and gull-specific source identifiers, HF183 and Gull4. C. jejuni (the most common cause of human campylobacteriosis) and C. lari (associated with gull feces) were also detected from most sampling locations. Genetic markers Rum-2-Bac and Pig-2-Bac specific for cattle and swine feces were present especially in the downstream sampling locations, from where also the most C. coli and Salmonella Typhimurium findings originated. Along the artificial groundwater production process, the last RNA-based GenBac3 RT-qPCR signal, representing active Bacteroidetes cells, was detected after the flotation and rapid sand-filtration before water infiltration into the sandy esker formation. Interestingly, the last DNA-based GenBac3 qPCR signal was detected from the one purification step further, from the first groundwater sampling tube indicating the persistence and transport of the Bacteroidetes DNA within the process. The microbiological findings support the previous observations of sewage discharges, scattered loading from agriculture and waterfowl feces as sources of fecal microbes including zoonotic pathogens like Campylobacter species and Salmonella serotypes into the surface water resources hampering the water quality. The measurements along in the Virtaaankangas artificial groundwater recharge plant producing drinking water for the city of Turku region showed that no pathogenic microbes could enter into the drinking water. In the future, we plan to investigate the land use patterns and the densities of cattle, swine and waterfowl at each region to further explain the findings. The relative influence of the upstream sewage effluent discharges on the surface water quality at each sampling location will be evaluated using the hydraulic transport models developed for the study area at the Finnish Environment Centre.

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**Impact of urbanization on physico-chemical and microbiological characteristics of Canals in Suzhou, China**

Sekar Raju, Xi’an Jiaotong-Liverpool University

Jonathan Tonkin; Jianjun Wang; Tianma Yuan; Zimeng Zhang; Kiran Kumar Vadde; Jing Zhang; Felicia Lim; Alan McCarthy
Urbanization is increasing worldwide and is happening at a rapid rate in China in line with the pace of economic development. Urbanization can lead to major changes to freshwater environments such as eutrophication, loss of biodiversity, and chemical and microbiological contaminations due to various industrial, domestic and agricultural point and non-point source discharges. Canals dominate the landscape of the Yangtze River floodplain in China, and canal water is often used for irrigation of horticultural crops, urban plantations and other domestic use. Therefore, it is important to ensure that these canals do not harbor high concentrations of chemical and biological contaminants, which can freely enter the food chain. The main aim of this study was to assess the water quality and microbiological characteristics of canal water in Suzhou across a gradient of urban intensification. Nine locations covering three urban intensity classes (High, Medium and Low) were sampled in four seasons (Spring and Summer 2014, Winter and Summer 2015) over a two-year period. Water samples were collected from each location for physico-chemical, microbiological (e.g. total viable count, TVC) and molecular (e.g. 16S rRNA gene copy numbers, a measure of total bacterial abundance) analyses. Multiple measures of nitrogen and phosphorus and TVC were significantly greater in high compared to medium and low urban intensity areas. However, the differences were less apparent between medium and low urban intensities. Significant seasonal variations were observed for several parameters, particularly nutrients. Temperature, pH, conductivity and 16S rRNA gene copy numbers did not show significant relationships with urban intensification, but a few of them varied seasonally. The overall results indicate that the urbanization does have an impact on water quality, particularly nutrients and viable bacterial counts. Further studies on the impact of urbanization on organic matter breakdown and microbial diversity patterns, which are currently in progress, might provide a better insight into the effects of urbanization on ecosystem function.

Efficacy Assessment of the Ecological Purification System use in Fiji

Maneesha Rao, University of the South Pacific

Treatment of drinking water is an integral aspect of the World Health Organisation (WHO) regulations. This continues to be a challenge for developing countries like Fiji where a considerable number of rural residents lack access to treated public water supply, evident from the re-emergence of typhoid cases in 2014. The present study has been conducted to assess the efficacy of the Japanese water intervention called the Ecological Purification System (EPS) which is used in some settlements of Fiji. The EPS is a modification of the traditional Slow Sand Filtration System with more emphasis on biological components such as algae, sunlight and oxygen as the added modes of contaminant removal from water. The study compares levels of total coliform, E. coli, Salmonella, TSS, BOD and pH in 100 ml of water before and after treatment by EPS. After treatment data analysis showed that 98% of the samples had non-detectable levels of E. coli where 50% of the water samples had E. coli ranging from 4 - 500 CFU/100ml before treatment. Significant removal (P = 0.03; 98% removal) of total coliform was also noted. TSS removal was insignificant (P = 0.632) with significant decrease in pH (P = 0.02) and insignificant difference to BOD (P = 0.35) in the water after treatment. Preliminary detection of Salmonella, was proved to be a false positive by confirmation tests. Hence, EPS is an effective device for removing coliform from water to safe levels. EPS effectiveness however, may vary depending on coliform population, contamination level of water, climatic patterns that may alter algal growth and the maturity and microbial population of the schmutzdecke (bio-layer).
Comparison of the qPCR and the HybriScan Legionella assays vs. culture for the identification of Legionella sp. in non-potable water samples

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Legionnaire's disease is a severe form of pneumonia caused by Legionella sp., with the majority of cases due to Legionella pneumophila. Mode of transmission includes the inhalation of contaminated, aerosolized water droplets from man-made water sources such as cooling towers, and aspiration of contaminated liquids. Outbreaks and sporadic cases of Legionellosis within communities have been attributed to contaminated, aerosolized water stemming from industrial buildings in close proximity to these communities. In order to evaluate and control the levels of Legionella sp. from these sources, constant monitoring of bacterial levels is recommended. Currently, the gold standard for identification and monitoring of Legionella sp. is by culturing samples onto selective and enriched media. However, this method for Legionella sp. identification has its drawbacks, including long incubation time (up to 10 days), inability to grow viable but non-culturable cells, and the difficulty in confirming serogroup or species. Therefore, it would be of benefit to apply methods for the detection of Legionella sp., in addition to the culture method, that will provide results earlier, and verify the identification of the organism. Quantitative PCR and the HybriScan Legionella assay are rapid methods for detecting Legionella sp. in water samples. Results from these assays can be obtained within the same day of processing. The qPCR method detects total Legionella DNA from the sample, but does not differentiate DNA from live vs. dead cells. The HybriScan assay detects Legionella rRNA by sandwich hybridization. Because rRNA is quickly decomposed in dead cells, the HybriScan assay detects only living cells. Both these methods are currently being employed, in addition to the culture method, by the Analytical Services department of the Northeast Ohio Regional Sewer District on boiler system samples obtained from the wastewater treatment plants. Results from these comparison studies, and their advantages, will be assessed.

Chicago Area Waterway System Microbiome Research Revealing Microbial Community Diversity and Abundance

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Cristina Negri; Jack Gilbert; Jarrad Marcell; Herbert Ssegane

The Metropolitan Water Reclamation District of Greater Chicago (MWRD) initiated a seven-year Chicago Area Waterway System (CAWS) Microbiome research in partnership with U.S. Department of Energy's Argonne National Laboratory (ANL). The study aims to document potential changes in CAWS microbial communities as the MWRD begins disinfecting its secondary treated effluents at the O'Brien and Calumet Water Reclamation Plants (WRP) in 2016 and as phases of the Tunnel and Reservoir Plan, the Thornton Composite Reservoir and the first phase of the McCook Reservoir are completed in 2015 and 2017, respectively. Water, sediment, WRP effluent and other targeted non-point source samples were collected and analyzed in the first three years (2013-15); additional samples will be collected each year until 2019 as the MWRD takes steps to improve the water quality. DNA isolation, 16S RNA
Monitoring for the human parasitic nematode Enterobius vermicularis in municipal wastewater effluent in Alberta, Canada

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Norman Neumann; Patrick Hanington

Demand for clean water has increased, in part due to population growth, climate change, and increased per capita water usage. Reuse of treated wastewater is essential to fill this demand for water; however public safety must be prioritized. Monitoring pathogen occurrence and fate during treatment is essential to assess risks associated with reuse. Often overlooked in North America are helminthic, or parasitic worm, ova. Certain populations—notably rural, first nations, or immigrants, are more likely to harbour such infections, and while the prevalence of worm infections in North America are largely unknown for many species, most parasitologists argue that infection rates are underestimated. Furthermore, as a single helminth infection may yield thousands of infectious ova per day, this amplification could pose a risk to certain wastewater reuse applications. Enterobius vermicularis, a parasite nematode that causes pinworm infection, is a good indicator for parasitic worm removal during the wastewater treatment process. Helminth ova are expected to settle out of the water matrix and into the biosolid phase during the primary and secondary sedimentation phases of treatment; however, this is largely dependent on the size, shape, and physicochemical properties. E. vermicularis is an interesting worm to study in wastewater as it represents a breadth of smaller, lighter helminth ova that are capable of aerosolization, and these ova may behave differently during treatment. We are currently monitoring for E. vermicularis presence in wastewater influent and effluent using a highly sensitive diagnostic qPCR assay. The assay is specific to the 5S rDNA gene of E. vermicularis. In addition to this detection test, we have also developed qPCR inhibition controls in the samples, and have also developed a parasite lysis control, using the muskrat trematode Echinostoma caproni. We find consistent levels of the parasite in wastewater influent, and most interestingly find that the parasite persists through flocculation/sedimentation and filtration through to discharge. This data will be used to perform a quantitative microbial risk assessment.
The changing face of water - shifting paradigms linking environmental antibiotic resistance to human health outcomes

Claire Sanderson, Virginia Tech

Eric Dougherty; Kathleen Alexander

The role of surface and ground water in the propagation of antibiotic resistance is absent from many surveillance systems and action plans aimed at combating antibiotic resistance emergence. These mediums, however, have the potential to act as optimal distributing and exposure routes, creating complex connections between and within divergent landscapes, and human and animal populations. We evaluate antibiotic resistance among Escherichia coli isolates (n=2082) collected bimonthly from surface water sampled across land use and season from the Chobe River in Northern Botswana (n=414; July 2011- May 2012). Municipal water is piped directly from the Chobe River to supply seven communities in the region. The vast majority of E. coli isolated from water samples were resistant to at least one antibiotic 80.9% (95% CI 79.2-82.6%), with over a quarter exhibiting multi-drug resistance (MDR; 25.5% (95% CI 23.6-27.4%)). MDR E. coli peaks occurred biannually in the Chobe River and were significantly correlated with diarrheal disease outbreaks among children under five years of age in this region ($r = 0.557; t = 2.684; p = 0.016$). MDR levels did not correspond to changes in water E. coli counts. Land use, season, and floodplain presence were significant predictors of antibiotic resistance levels. The apparent coupling of surface water MDR peaks with diarrheal disease outbreaks among children in the region identifies a paradigm shift, linking environmental processes and MDR E.coli with human health outcomes. Our results emphasize the dynamic nature of resistance in surface water microbial populations and suggest water and interacting environmental drivers influence the movement of antibiotic resistance across the landscape. Rather than focusing sentinel surveillance on the emergence of antibiotic resistance in human and animal pathogens, as currently proposed in global action plans, perennial surface water resources identify an ecosystem level surveillance system, where environmental antimicrobial hot spots can be identified and scaled investigations launched, before dissemination into clinically significant pathogens.

qPCR: A Screening Tool For Harmful Algal Blooms

Nicki Schafer, NEORSD

Lake Erie has seen an increase in the number of harmful algal blooms (HAB) caused by cyanobacteria (blue-green algae) as well as an increase in the severity of these blooms. The cyanobacteria present in the HABs can potentially produce toxins capable of causing illness and/or death. Timely and accurate identification and reporting of these toxins is critical for issuing water quality advisories. The analytical methods for toxin analysis are very expensive and selecting the correct analytical method can be difficult. Another challenge is the necessity for a skilled analyst available for microscopic algae identification. Furthermore, some cyanobacteria can produce multiple toxins. For example the genus Anabaena can produce anatoxin-a, saxitoxin, or microcystin and the genus Aphanizomenon can produce saxitoxin, anatoxin-a, or cylindrospermopsin. NEORSD decided to experiment with a rapid method utilizing Quantitative Polymerase Chain Reaction (qPCR) to screen for a total cyanobacteria gene and specific toxin producing genes (microcytins, saxitoxin, and cylindrospermopsin). The NEORSD
laboratory experimented with the Phytoxigene, CyanoDTec qPCR assays as a means to screen samples submitted for cyanotoxin analysis. A portion of the sample submitted for analysis was filtered, and the DNA was extracted and analyzed on multiple qPCR platforms. The results of our study indicate that this method has the potential to eliminate the need for microscopic examination and assist with the selection of the appropriate method for toxin analysis.

EPA Method 1603 "Spike the Ball" Study

Nicki Schafer, NEORSD

EPA Method 1603: "Escherichia coli (E. coli) in Water by Membrane Filtration using Modified membrane-Thermotolerant Escherichia coli Agar (Modified mTEC)," requires the use of a spike of a known value to test "Ongoing Precision and Recovery" (OPR), and "Matrix Spikes" (MS). The method proposes the use of a manufactured E.coli strain called a "BioBallTM," of known concentration, or the use of a lab-prepared E.coli strain. The lab-prepared E. coli strain has an optimal incubation temperature of 35-37°C and the manufactured BioBallTM has an optimal temperature incubation of 35-37°C. Previous studies in the lab demonstrated a decrease in recovery when a laboratory E.coli strain was incubated at method temperatures, 2 hours at 35°C, and 22-24 hours at 44.5°C (the temperature at which thermo-tolerant E. coli grow). The increased temperature drastically reduced the count and viability of the colonies. Additional studies in the lab using the BioBallTM spike showed a decrease in recovery when the BioBallTM was plated onto Modified mTEC Agar. The decrease was a result of the inhibitory properties of the modified mTEC agar, and the increased incubation temperature at 44.5OC. The Analytical Services department of the Northeast Ohio Regional Sewer District initiated a study for alternative spiking procedures for Method 1603 in order to resolve the limitations of the current bioball procedure. The spiking procedure was developed using an environmental thermo-tolerant strain of E. coli. Percent recoveries and RSD were assessed. A percent recovery of 83% to 110%, and an RSD of 9.6% validates the spikes and confirms repeatability. The recovery range of the thermo-tolerant spikes approached 100% recovery, whereas the BioBallTM was at 26%. This alternative approach to spiking demonstrated higher efficiency by eliminating the analysis of lab-prepared suspensions for every single spiking requirement, and lowered costs of testing by the use of a laboratory isolated strain rather than a manufactured strain, such as the BioBallsTM. A further advantage to our method is its ability to indicate any matrix interference as compared to the other two protocols which provide wide and unreliable recovery ranges by using E. coli strains that cannot grow favorably at 44.5 oC and/or on a selective media such as modified mTEC agar.

Make It "Tough": qPCR Master Mix Comparison

Nicki Schafer, NEORSD

Within the 2012 Recreational Water Quality Criteria guidelines the EPA provides information for states who want to adopt Water Quality Standards based on rapid methods that EPA has developed and validated using quantitative polymerase chain reaction (qPCR). Currently the EPA has validated Method 1609 and 1611 for the quantification of Enterococcus in environmental waters using qPCR. The NEORSD laboratory has collected samples from two Northeast Ohio Beaches and analyzed them by...
EPA method 1609 and a qPCR assay for E. coli. The objectives of this study was to identify a master mix that (1) could be used for both assays (2) reduce inhibition (3) is similar to the Environmental Master Mix (EMM) recommended by the EPA (4) utilizes an Internal Amplification Control (IAC) allowing for analysis on the same platform and (5) contains a purified DNA polymerase that is validated free of extraneous E. coli DNA. A total of 241 samples were analyzed for both organisms using two different chemistries, EMM as stated in method 1609 and Tough Mix. The data for each organism and master mix combination was compared for inhibition, method QC, level of quantification and difference between assay absolute and relative quantitation. The accuracy, sensitivity and specificity were calculated as compared to the conventional method. In conclusion, we have determined that the Tough Mix yielded comparable data to the EPA recommended mix with respect to the accuracy and reproducibility but the level of inhibition and instance of positive QC data decreased with the Tough Mix.

Performance Characteristics Of Human Host-Associated Escherichia coli Markers In Microbial Source Tracking In Florida

Jacob Senkbeil, University of South Florida

Valerie Harwood

Fecal pollution of ambient waters increases the risk of waterborne transmission of fecal-derived pathogens to human populations. In order to limit human health risks, regulatory agencies quantify fecal indicator bacteria (FIB) as a measure of fecal contamination. FIB, including Escherichia coli and enterococci, are commensal bacteria found in the gut flora of all animals which are shed in the feces. Without identification of fecal sources, accurate assessment and remediation of human health risks is a difficult task. In this study five human host-associated E. coli markers (H8, H12, H14, H24, & ycjM) were assessed for their performance characteristics (host-specificity, host-sensitivity, and positive/negative predictive values) in microbial source tracking of E. coli in Florida. A total of 180 E. coli colonies isolated from target (human), and 900 E. coli colonies isolated from non-target (cat, cow, dog, duck, feral pig, alligator, and horse), host groups were tested for marker presence using conventional PCR. Host-sensitivity values, the percentage of target E. coli isolates tested PCR positive, were 17%, 6%, 19%, 13%, and 5% for H8, H12, H14, H24, and ycjM. Host-specificity values, the percentage of E. coli colonies isolated from non-target host groups PCR negative for these markers, were 99%, 99%, 99%, 93%, and 97% for H8, H12, H14, H24, and ycjM. Positive predictive values, the number of target E. coli isolates correctly classified as human host-associated divided by the total number of target and non-target E. coli isolates tested PCR positive for marker presence, were 91%, 59%, 80%, 30%, and 31% for H8, H12, H14, H24, and ycjM. Negative predictive values, the number of non-target E. coli isolates correctly classified as PCR negative for marker presence divided by the total number of target and non-target samples tested PCR negative for marker presence, were 85%, 84%, 86%, 85%, and 84% for H8, H12, H14, H24, and ycjM. Based on host-specificity and positive predictive values, H8 and H14 markers show the greatest promise for use in microbial source tracking of human host-associated E. coli in Florida, while the low host-sensitivity and positive predictive value of H12, H24, and ycjM make them unsuitable for microbial source tracking applications. In the future, assessment of the qPCR-based performance characteristics of H8 and H14 in comparison to currently used MST assays, including
HF183 and PMMoV, would provide a valuable insight into the relative usefulness of these markers.

A human fecal contamination index for ranking impaired recreational waters using the HF183 quantitative real-time PCR method

Orin Shanks, US EPA
Yiping Cao; Mano Sivaganesan; Catherine Kelty; Dan Wang; Alexandria Boehm; John Griffith; Stephen Weisberg

Human fecal pollution of surface water remains a public health concern worldwide. As a result, there is a growing interest in the application of human-associated fecal source identification quantitative real-time PCR (qPCR) technologies for recreational water quality risk management. The transition from a research subject to a management tool requires the integration of standardized water sampling, laboratory, and data analysis procedures. In this study, a standardized HF183/BacR287 qPCR method was combined with a water sampling strategy and Bayesian data algorithm to establish a human fecal contamination index that can be used to rank impaired recreational water sites polluted with human waste. Stability and bias of index predictions were investigated under various parameters including sites with different pollution levels, sampling period time range (1-15 weeks), and number of qPCR replicates per sample (2-14 replicates). Sensitivity analyses were conducted with simulated data sets (100 iterations) seeded with HF183/BacR287 qPCR laboratory measurements from water samples collected from three Southern California sites (588 qPCR measurements). Findings suggest that site ranking is feasible and that all parameters tested influence stability and bias in human fecal contamination index scoring. Trends identified by sensitivity analyses will provide managers with the information needed to design and conduct field studies to rank impaired recreational water sites based on levels of human pollution.

Virus survival in produce under storage and process environments

Carol Shieh, US Food and Drug Administration

Enteric viruses are the leading cause of foodborne infections in the U.S. The greatest number of foodborne illnesses is attributed to norovirus (NoV). Hepatitis A virus (HAV) is also a significant contributor, due to the hepatitis disease severity. Virus-contaminated water and food are major vehicles for transmitting the diseases. Fresh produce is the top commodity of foods linked to viral gastroenteritis. The virology program at FDA Center for Food Safety and Applied Nutrition Moffett Campus has focused on post-harvest control research to evaluate virus inactivation in produce before the product reaches the consumer. Green onions, berries and pomegranates, which were potentially contaminated in the field or during the harvest, have been associated with viral outbreaks and were chosen for the studies. In onions, the decimal reduction time of HAV was 30, 14, 11, and 5 days during storage at 3, 10, 14, and 23°C, respectively. These results imply that HAV survives well beyond the shelf-life of fresh produce. Common food-preserving procedures of low thermal dehydration, freeze-drying, and high hydrostatic pressure process (HPP) were also examined for their capability to inactivate viruses in produce. During a 20-hr dehydration of onions involving thermal treatments of 48,
55, or 62, HAV was reduced by 1, 2, and 3 logs, respectively. During 24 hr of freeze-drying of strawberry slices, both NoV and HAV survived with 38 -radiant heat and reduced atmospheric pressure. Conversely when high pressure (300-600 MPa) was incorporated, HPP increased the kill of both viruses as the pressure increased. A 5-log reduction of murine NoV in strawberry puree was achievable at 424 MPa for 3 min using 20 process-holding temperature. However, HPP holding-temperature (0 to 38 ) presented drastically different impacts on the survival of the two virus groups. This research provides data that can support possible control options for reducing NoV and HAV in food and water.

Survival and Disinfection of Ebola Virus Surrogates in Hospital Sewage and Human Fecal Samples

Emanuele Sozzi, UNC

Joe Strasser

Survival and Disinfection of Ebola Virus Surrogates in Hospital Sewage and Human Fecal Samples
Joseph Strasser, Dr. Emanuele Sozzi and Dr. Mark Sobsey Background Feces and other body fluids from Ebola cases in healthcare facilities in the U.S. can be discharged directly to local sewage systems for treatment, according to CDC guidance. However, this may put sewer workers and others at risk. Therefore, research is needed urgently to determine the survival of Ebola virus and surrogates for it in human sewage and fecal wastes and the ability of chemical disinfectants to inactivate these viruses in such wastes. Aim of the research The major goals of the project are to provide critical and timely quantitative information on the survival and rapid on-site chemical disinfection of Ebola virus through a detailed study performed using several candidate indicator/surrogate viruses in feces and raw sewage and, at a later stage, a mutant Ebola virus (ΔVP30). Material and methods The methodology for the assessment of the survival and rapid on-site chemical disinfection of the viral surrogates mentioned is outlined on following diagram. For the survival experiments the kinetics and extent of inactivation of surrogate/indicator viruses within the fecal waste matrix was quantified every 24 to 48 hours until it was possible to accurately assess the kinetics of inactivation. The kinetics and extent of inactivation of the infectivity of surrogate/indicator viruses within the fecal waste matrix by the chemical disinfectants was quantified for contact times of 1, 3, 10 and 30 minutes. A 5 log10 (99.999%) virus reduction was considered as the threshold requirement to achieve low risks of remaining infectious viruses. Results Survival experiments were conducted on both MS-2 and PhiX-174 coliphages non-enveloped viruses and Phi-6 bacteriophage enveloped virus at ambient room temperature (~23 oC). MS-2 coliphage reduction was 0.55 log10 in unpasteurized hospital sewage over a 16-day period. PhiX-174 showed a 1.2 log10 reduction over the same 16-day period under the same conditions. Comparatively, MS-2 showed a 2.7 log reduction over an 18-day period in a more realistic unpasteurized matrix of 25% human feces and 75% hospital sewage and PhiX-174 displayed a 1.0 log reduction in this matrix over the same period. Enveloped Phi-6 bacteriophage experienced greater but somewhat more variable inactivation in both matrices compared to the non-enveloped coliphages. In unpasteurized hospital sewage one trial resulted in complete inactivation (7.6 log reduction) in 3 days and another trial resulted in complete inactivation (8.8 log reduction) in 6 days. Phi-6 experienced far lower inactivation in the unpasteurized matrix of 25% human feces and 75% hospital sewage, exhibiting only a 4.7 log reduction over 18-days. Chlorine disinfection experiments are in progress in the more realistic 25% human feces and 75% hospital sewage matrix and results will be reported in the
Development of Cross-Assembly Phage PCR-Based Methods for Human Fecal Source Identification

Elyse Stachler, University of Pittsburgh

Catherine Kelty; Kyle Bibby; Orin Shanks

Technologies that can characterize human fecal pollution in environmental waters offer many advantages over traditional general indicator approaches. However, many human-associated methods cross-react with non-human animal sources and lack suitable sensitivity for fecal source identification applications. The genome of a newly discovered bacteriophage (~97 kbp), the Cross-Assembly phage or “crAssphage”, assembled from a human gut metagenome DNA sequence library is predicted to be both highly abundant and predominately occur in human feces suggesting that this double stranded DNA virus may be an ideal human fecal pollution indicator. We report the development of two human-associated crAssphage endpoint PCR methods (crAss056 and crAss064). A shotgun strategy was employed where 384 candidate primers were designed to cover ~41 kbp of the crAssphage genome deemed favorable for method development based on a series of bioinformatics analyses. Candidate primers were subjected to three rounds of testing to evaluate assay optimization, specificity, limit of detection (LOD95), geographic variability, and performance in environmental water samples. The top two performing candidate primer sets exhibited 100% specificity (n = 70 individual samples from 8 different animal species), >90% sensitivity (n = 10 raw sewage samples from different geographic locations), LOD95 of 0.01 ng of total DNA per reaction, and successfully detected human fecal pollution in impaired environmental water samples. Data suggests that novel crAssphage genetic indicators are highly abundant in raw sewage, closely associated with human fecal pollution, and readily detectable in impaired environmental waters showing great promise for future fecal source identification applications. This presentation will discuss the design and testing methodology used to identify human-associated crAssphage genetic regions and present evidence warranting future water quality monitoring research. Disclaimer: This abstract does not necessarily reflect U.S. EPA policy.

Quantification of Pathogenic Viruses, and Bacteria in Storm Water Discharging to Beaches with Year-round Surfer Populations in San Diego, California

Joshua Steele, Southern California Coastal Water Research Project

Presentation. Conclusions The preliminary results from this study on survival and rapid chemical disinfection of Ebola and other surrogate/indicator viruses in feces and raw sewage are currently informing the design of effective on-site management systems and protocols in healthcare and other settings to reduce the risks of the spread of Ebola and other high risk viruses fecally shed by people sick with the diseases they cause. Initial results from experiments comparing the two different fecal waste matrices suggests that a matrix including 25% human fecal sample and 75% hospital sewage better represents what would have to be disinfected during a real future epidemic. At this stage, it is reasonable to say that a matrix exclusively made of more dilute hospital sewage would not be sufficiently representative to adequately reflect the real case scenario that begins with excreted feces. Obtaining quantitative data for a worse case fecal waste sample and scenario is preferred for estimating virus survival risks and the efficacy of chemical disinfection processes.
Kenneth Schiff; A. Denene Blackwood; Emelie Andersson; Rachel Noble; John Griffith

California receives the bulk of its rainfall during winter months, and has year round surfer populations at many of its beaches. Water quality at these beaches worsens during and following rainstorms with dramatic increases in fecal indicator bacteria (FIB) concentrations, leading to well known 72-hour rain advisories. These advisories were originally established using the relationship of FIB concentrations to pathogens in raw sewage, but the relationship between FIB concentrations and pathogens in stormwater has not been sufficiently explored. Further, the pathogenic bacteria and viruses in stormwater that likely cause illness have historically been measured using molecular approaches that are not sensitive enough to accurately quantify dilute pathogens in complex water matrices. Digital PCR now offers the sensitivity to enable direct quantification of pathogenic viruses and bacteria, without the impacts of inhibition. Using traditional culture methods and digital PCR assays, we measured microbial water quality in wet weather stormwater discharges at two beaches in San Diego, CA that are frequented by large, year-round surfer populations. Tourmaline Creek drains a small urban watershed and the San Diego River drains a large mixed urban/undeveloped watershed. Stormwater samples were collected during six events with total precipitation ranging from 0.19-2.5” from January-March 2014 and December 2014-March 2015. Stormwater composite samples were collected during the rain event, and grab samples were collected over 72 hours following the beginning of rainfall. Enterococcus spp., and total and fecal coliform concentrations were measured in Tourmaline Creek and San Diego River discharge and samples were also taken at proximal beaches. Previously published and validated molecular markers specific to human, dog, and bird feces were quantified in the stormwater using digital PCR. Pathogens in stormwater were directly quantified with digital PCR for Campylobacter (including C. jejuni, C. coli, and C. lari), Salmonella, human norovirus GI and GII, human adenovirus, and human enterovirus. In every storm measured, we found high FIB and pathogen concentrations in both the large and small watershed stormwater during the storms and in the 3 days following rainfall. Molecular source tracking markers revealed dog and bird fecal sources in addition to human sources in the stormwater, but concentrations along the beaches were diluted. Human norovirus genotypell and Campylobacter spp. were detected most often, while Salmonella and human adenovirus were rarely detected, and enterovirus was not detected. We found no consistent relationships between FIB and pathogen concentrations. FIB concentrations measured at the beach decreased with distance from the mouth of the San Diego River, but did not change with distance from Tourmaline Creek, reflecting differences in the dispersal of the stormwater plume by waves and ocean currents at each site. The markers and pathogens measured suggest multiple sources of microbial pollution within the San Diego River and Tourmaline Creek watersheds. The ability to quantify pathogens, even at low concentrations, in stormwater provides the ability to more precisely estimate the risk of illness to public health.

Analysis of fecal indicator data to identify periods of microbial challenge in drinking water treatment plants

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Jean-Baptiste Burnet; Sarah Dorner; Michèle Prévost
Background: Drinking water treatment plants (DWTPs) located in urban areas are vulnerable to rapid fluctuations of microbial water quality, notably following heavy rainfalls. Several studies have demonstrated that these critical events can increase significantly the cumulative risk of waterborne gastrointestinal illness. In order to manage that risk, surface water monitoring should be aimed at achieving a representative quantification of pathogenic microorganisms in the source water. However, given that pathogen concentrations can vary substantially either seasonally or over short-term, monitoring strategies need to capture the peak events at DWTP intakes, which will improve the assessment of the associated microbial risk and in the identification of fecal contamination sources.

Objectives: 1) Assessing the extent of temporal variability of fecal contamination at drinking water intakes (DWI) located in highly urbanized areas, 2) Evaluating the potential of high-frequency event based sampling of fecal contamination to assess short-term fluctuations in microbial water quality, 3) Examining the suitability of international regulations on source water protection (New Zealand, USA, Quebec) to assess microbiological quality. Methodology: Seasonal variability was evaluated by analyzing weekly E. coli monitoring at 11 DWI from 2013 to 2015 in highly urbanized areas from the greater Montreal. Short-term fluctuations in E. coli were examined at one DWI by collecting samples 4 days a week over 2 years (2014-2015). Finally, rainfall-induced peak events will be sampled during spring 2016 with an online auto-sampler capable of measuring E.coli every 15 minutes. Results: The median value, which is usually recommended by regulations, is highly variable depending on the season and is not reliable to consider peak values. An average increase of 136% between the median and the arithmetic mean shows the relative importance of peak values. Distributions are highly skewed (average increase of 120% between the 95th and 99th centile) and are not accurately defined by weekly sampling. Over short term, 95th centile increases of 260% during the wet season (March to April). Therefore, high-frequency sampling during heavy rainfall events will allow to better characterize amplitude and duration of peak events. Finally, preliminary results indicate that vulnerability indexes proposed by regulators are discordant and not able to account for short-term fluctuations. Relevance: A better understanding of temporal variability in microbial quality in source water is required to implement effective multi-barrier strategies. This study will provide unique data that should ultimately improve quantitative microbial risk assessment, the identification of fecal contamination sources, and support the development of early warning systems for DWTP.

**Incidence of Somatic and F+ Coliphage at Three Great Lake Beaches**

Pauline Wanjugi, US EPA, ORD

Mano Sivaganesan; Catherine Kelty; Asja Korajkic; Eric Rhodes; Brian McMinn; Mike Cyterski; Kevin Oshima; Elyse Stachler; Ariela Topper; Lucas Bertaux-Skeirik; Julie Kinzelman; Mark Citriglia; Fu-Chih Hsu; Orin Shanks

There is a growing interest for the potential use of coliphage as an alternative indicator to assess fecal pollution in recreational waters. Coliphage are a group of viruses that infect E. coli and are commonly used as models to infer the likely presence of human enteric viral pathogens. However, many uncertainties still exist concerning the application of coliphage for recreational water quality monitoring. We report the use of a dead-end hollow fiber ultrafiltration single agar overlay method to enumerate F+ and somatic coliphage from surface waters collected from three Great Lake beaches and nearby discharging rivers with historically high E. coli densities. At each beach location, three sites (two
beach; one river) were sampled five days a week over the 2015 beach season (n = 580 total samples). In addition, E. coli and enterococci densities, as well as 16 physical, chemical, climate and beach condition parameters were assessed such as rainfall, turbidity, dissolved oxygen, pH, ultra violet irradiation and number of birds. Overall, somatic coliphage levels ranged from non-detectable (ND) to 4.39 log10 PFU/L and were consistently higher compared to F+ (ND to 2.84 log10 PFU/L), regardless of water sample. Both somatic and F+ coliphage were significantly correlated with E. coli (MPN/100mL) and enterococci (CFU/100mL) densities (p < 0.05). Distinct spatial and temporal trends in coliphage densities were apparent based on sampling site location (within and between beaches), rainfall, and occurrence of combined sewer overflow events. In addition, results indicate that some physical and chemical properties are more closely correlated with coliphage densities than others suggesting additional research on the development of forecasting models is warranted.

**Determination of adsorption and desorption of general Bacteroidales genetic marker on freshwater and marine sediments by quantitative real-time PCR**

Jia Xue, Auburn University

Yucheng Feng

Adsorption of DNA by sediment increases the persistence of free DNA in the aquatic environment and thus causes ambiguity for the identification of recent fecal pollution sources when nucleic acid based methods are used. In this study, the adsorption and desorption of DNA molecules on both freshwater and marine sediments were quantified using real-time PCR. A minimum of 36 hours was needed for sorption to reach equilibrium. Both DNA extracted from raw sewage and purified PCR products were used in the experiment and their sorption kinetics showed different trends. More DNA was adsorbed on both sediments in stream water than in 5 mM NaCl solution. DNA adsorption on both sediments was increased in the presence of Mg2+ and Ca2+. Clay content in the sediments was another important factor influencing DNA adsorption capacity. Adsorption data were fitted with the Langmuir and Freundlich equations. The observed DNA adsorption capacity was higher than the maximal capacity estimated from the Langmuir equation, suggesting the presence of multilayer adsorption. Desorption experiments were performed using various solutions and 5-22% of adsorbed DNA was desorbed. The results indicate that more DNA molecules were adsorbed on sediment through ligand binding than electrostatic binding.

**Fate and Recovery of Enveloped Viruses in Municipal Wastewater**

Yinyin Ye, University of Michigan

Krista Wigginton

A number of enveloped viruses have emerged to cause global panic in recent years (e.g., SARS coronavirus, avian influenza virus, Ebola virus). Although not usually associated with water transmission, the genes of enveloped viruses, including coronaviruses and avian influenzas, have been detected in the human excrement and wastewater samples. Relatively little is known about the
presence and fate of enveloped viruses in the aqueous environment compared to non-enveloped enteric viruses. In this presentation, we will present our research on enveloped virus fate and recovery in untreated wastewater. In particular, we compared the survival and partitioning behavior of two enveloped viruses (Murine hepatitis virus, MHV and bacteriophage Φ6) and two non-enveloped bacteriophages (MS2 and T3) in raw wastewater samples collected from a local wastewater treatment plant. Our results demonstrate that enveloped viruses are inactivated faster than non-enveloped viruses, yet survive long enough to be of public concern, especially in cool climates. Virus adsorption and inactivation modeling based on our experimental data showed that enveloped viruses adsorbed to wastewater solids to a greater extent than non-enveloped viruses. Based on our partitioning results, we developed an extraction/concentration method for recovering infective enveloped viruses from wastewater. The optimized ultrafiltration method resulted in mean virus recoveries of 25.1% for MHV, 18.2% for Φ6, 55.6% for MS2 and 85.5% for T3. The results presented here will be valuable in future outbreaks of deadly enveloped virus diseases, and will aid research focused on enveloped viruses in aqueous environments.

Survival of microbial source tracking markers, pathogens and antibiotic resistance genes in poultry litter microcosms

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B. Nayak; J. Weidhaas; V Harwood

Agricultural runoff contributes to fecal pollution and spread of antibiotic resistance in the environment. Methods of tracking the sources and survival of microbial pollutants entering aquatic ecosystems can be assessed and validated in ecologically relevant microcosm studies. Microbial source tracking (MST) uses genetic markers unique to the gut microbiome of different animal species to identify sources of fecal pollution. Here, experimental microcosms were constructed to assess the survival of MST markers, pathogens and antibiotic resistance genes (ARGs) associated with poultry litter over seven days in two seasons. Water and sediment from freshwater and marine sources in Tampa Bay were used as substrate and inoculated with poultry litter from West Virginia. Bacteria (E. coli, enterococci, Bacteroidales, Salmonella enterica, C. jejuni, C. coli), MST markers (Brevibacterium sp. LA35) and six ARGs (tetB, tetM, qnrS, ampC, aadA, ermB) were measured over seven days, and in the initial substrates of water, sediment and poultry litter inoculum. Bacteria and MST markers decreased over time in water, but persisted and even increased over time in sediment, especially in marine sediments. LA35 correlated with other bacteria in water (P < 0.05) and covaried with bacteria, including fecal indicators, independently of seasonal differences, indicating that it is a useful marker for tracking and detection of poultry associated fecal pollution. Genes for Class-1 integrons (ampC, aadA), and genes encoding resistance to tetracycline (tetB, tetM) and erythromycin (ermB) were detected by PCR in winter microcosms and persisted for 4-7 days in water and sediment in both marine and freshwater microcosms. Genes encoding resistance to fluoroquinolones (qnrS) were not detected by PCR. Poultry litter used to inoculate the winter microcosms also contained all ARGs measured except for qnrS. Antibiotic resistance genes can reach environmental reservoirs through poultry litter and persist in simulated microcosms for up to seven days. Pathogens and ARG can enter the environment through waste from poultry farming and should be assessed in management of watersheds and
Development and validation of a real-time PCR assay to detect infectious F+DNA coliphages in water following rapid enrichment

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Mark Sobsey

Background: Coliphages are suggested indicators for enteric viruses in water quality monitoring because of their morphological and source similarities. Both are prevalent in wastewater and the intestinal tracts of humans and warm-blooded animals. Coliphages are preferred candidates for rapid detection methods because thousands of progeny phages can be produced in a short period in a bacterial host and then be detected by molecular methods. Several qRT-PCR methods are available for F+RNA coliphages because of their potential usefulness for contaminant source tracking but none have been developed for F+DNA coliphages. Base on our previous studies, F+DNA coliphages were detected in more than 98% of marine water samples positive for the presence of F+ coliphages, far greater than F+ RNA coliphage detection. Therefore, not having a molecular assay available for detecting F+DNA coliphages hinders their possible use as a rapid virus indicator for water quality monitoring. Objectives: To evaluate the effectiveness of five different growth media for rapid F+DNA coliphage propagation in E. coli host cells before downstream real-time PCR detection; and to developed and validated a SYBRGreen real-time PCR assay for detecting F+DNA coliphages in water and enrichment. Methods: Rapid enrichment: Five liquid enrichment media, Tryptic Soy Broth (TSB), Lauria Broth (LB) and Minimal 9 (M9) with glucose, lactose or glycerol, were evaluated for F+DNA coliphage rapid propagation. One-liter volumes of test water were spiked with 1-9 PFU of F+DNA coliphages (M13, fd and f1) and then 109 CFU Famp E. coli log phase host, 50mL of 10X enrichment media, 12.5mL of 4M MgCl2 and 10mL of 100X streptomycin and ampicillin stock were added. The samples were mixed vigorously and then incubated in a 37°C water bath. The growth of coliphages was measured hourly for the first 6 hours and then after 24 hours. Samples taken after 2, 3 and 4 hours of incubation were used for real-time PCR. Two DNA extraction methods were evaluated: (1) 25uL of samples were heated for 5-minute at 98°C and (2) 100uL of samples were lysed with buffer then DNA extracted with silica columns. F+DNA qPCR development: Complete genomic sequences of F+ DNA coliphages M13, fd and f1 were aligned using Clustal Omega. The consensus region in the G3P attachment protein was used for primers development. The annealing temperature and primer concentrations were optimized using a gradient of conditions for them. The new assay was tested with 123 F+ coliphage plaques picked from marine water samples. Results were compared to the RNase sensitivity assay and a subset of samples was sequenced. Results: F+DNA coliphage strains fd and f1 grew faster than M13. For fd and f1 after 3 hours of incubation, >5-log growth was achieved in LB and M9 with glucose enrichments, and ~4-log growth in TSB enrichment. M13 almost reached 5-log growth after 3 hours of incubation in M9 with glucose enrichment but concentrations were only ~3-log for TSB and LB enrichments. M9 with lactose or glycerol produced significantly less progeny phages than TSB, LB or M9 with glucose. The F+DNA coliphage real-time PCR assay annealing temperature (58°C) and primer concentrations (500nM) were optimized using f1. The amplification efficiency and detection limit for M13, fd and f1 were 104% and 4PFU, 100.6% and 1.4PFU, and 91% and 0.9PFU, respectively. Real-time PCR results from 123 F+ coliphage plaques were compared to the RNase sensitivity assay, with 82.1% positive for both, 4.1%
negative for both, 8.1% positive for real-time PCR but negative for RNase sensitivity and 5.7% negative for real-time PCR but positive for RNase sensitivity. Conclusions: LB or M9 with glucose are the most effective enrichment media for rapid F+DNA coliphage propagation in E. coli. This new real-time PCR method for detecting F+DNA coliphages and existing qRT-PCR methods for F+RNA coliphages can be coupled with rapid enrichment to detect all F+ coliphage in water in <5 hours. This rapid method can improve water quality monitoring for F+ coliphages to support their development as fecal indicator viruses for recreational water quality management.

**Enhanced detection of poliovirus in environmental samples from Kenya using the bag-mediated filtration system**

Nicolette Zhou, University of Washington

Christine Fagnant; Jeffry Shirai; Nicola Beck; James Nyangao; Evans Komen; Benlick Mwangi; Walda van Zyl; Marianne Wolfaardt; Maxime Muilwijk; Peter Matsapola; Fhatuwani Ngwana; Angela Coulliette-Salmond; Stacey Jeffries-Miles; Silvia Penaranda; Cara Burns; Peter Borus; Maureen Taylor; J. Scott Meschke

Environmental surveillance of poliovirus (PV), as a supplement to acute flaccid paralysis surveillance, plays an important role in the global program for eradication of wild PV (WPV). Environmental surveillance has been used to document the elimination of WPV in some areas, has shown the resurgence of WPV in previously polio-free areas, and will be central for monitoring the disappearance of the Sabin-like PVs as the live attenuated, oral polio vaccine (OPV) is replaced by the inactivated polio vaccine (IPV). The bag-mediated filtration system (BMFS) is a newly developed technology for enhanced environmental PV surveillance. The objective of this study was to test the applicability of the BMFS for monitoring PV in environmental samples. BMFS samples and grab samples (collected according to World Health Organization (WHO) guidelines and concentrated using the two-phase polyethylene glycol (PEG)/dextran method) were collected from four sites in Nairobi, Kenya (March to September 2015) and compared side-by-side. BMFS sample concentrates (n=56) were analyzed for Sabin-like PV types 1, 2, and 3 (SL1, SL2, and SL3) by direct real-time RT-PCR and by integrated cell culture real-time RT-PCR after amplification on three cell lines (PLC/PRF/5, L20B, and BGM). Grab sample concentrates (n=44) were analyzed for SL1, SL2, and SL3 by virus isolation using L20B and RD cells followed by intratypic differentiation using the real-time RT-PCR method recommended by the WHO. PV was detected more frequently in the BMFS samples after amplification in cell culture (79%) than by direct real-time RT-PCR (45%). PV was detected in the environmental samples more frequently in the BMFS samples (82%) than in the grab samples (66%). SL2 was the most frequently detected serotype (63%), followed by SL3 (46%), and SL1 (26%) in all samples (n=100). This will provide an important comparative baseline once the trivalent OPV is switched to bivalent OPV in April 2016, with the removal of PV-2 from the vaccine. Thirty-three samples have SL1, SL2, and SL3 data available for both BMFS and grab samples. Of these samples, 30 BMFS samples were positive for one or more serotype, 24 grab samples were positives for one or more serotype, and 10 samples were discordant between sample types. The greater frequency of detection in the BMFS samples is likely due to the greater volumes filtered with the BMFS (2.9 ± 0.4 L) compared to the 1 L grab samples. After processing, the BMFS samples are concentrated 3000 times and the grab samples 33 times. As 3 mL of both sample types are inoculated into tissue culture, this results in a greater effective volume assayed
of the original sample (900 mL BMFS vs. 100 mL grab sample). Differences in detection frequency between the BMFS and grab samples could also be due to the different real-time RT-PCR methods used and their sensitivity. This study demonstrated that the BMFS is applicable for environmental sampling of PV, and suggests that it could result in enhanced detection of PV, though further evaluation is needed, including parallel testing of the two methods.
POSTER PRESENTATIONS
Feasibility of Escherichia coli as an indicator of the electrochemical disinfection with a BDD anode

Rosa Maria Araujo Boira, Universitat de Barcelona

C Bruguera; I Sires; E Brillas

The electrochemical disinfection has gained much progress in recent years as a water disinfection technology characterized by easy and mild operation conditions, as well as environmental compatibility due to the in-situ production of oxidants without requiring the addition of noxious chemicals. The most relevant parameter in the electro-oxidation of water contaminated with bacteria is the anode material, being boron-doped-diamond (BDD) the most promising one. Most of published literature on electrochemical disinfection has been focused on Escherichia coli, because it has been accepted as an indicator of the process, although it does not exist any work demonstrating that this bacteria is an adequate surrogate for other type of bacteria. Therefore, it should be tested if E. coli is an adequate indicator of the electrochemical disinfection process on different type of bacteria, in particular, those with different cell wall. The objective of this work was to assess the bactericidal effect of the oxidants produced by electro-oxidation using a BDD anode on two bacteria: Escherichia coli and Bacillus subtilis, with different cell wall. Aqueous samples artificially contaminated, either with Escherichia coli or Bacillus subtilis suspended in 100 ml of 7 mM Na2SO4, were electrolyzed in a tank reactor equipped with a BDD anode and a stainless steel (AISI 304) cathode of 3 cm2 geometric area. A decrease of more than 6 log in bacterial cultivability was obtained after 45 min for E. coli and 30 min for B. subtilis when electrolyzing the solutions at a constant current density of 33.3 mA cm-2. This significant bacterial inactivation could be achieved with a BDD anode due to the main action of the physisorbed BDD(?OH) radical formed on its surface from the anodic oxidation of water. In the case of E. coli, a shoulder was observed, whereupon the decrease progressed up to overall disinfection. The opposite behavior was found for B. subtilis, which underwent a quicker inactivation from the beginning of the electrolysis. The observation of the cells before and after treatment by means of the Life and Dead staining method showed that most of cells presented unaffected membranes (green color) before the treatment, whereas the cells membranes became damaged (red color) after 45 min of treatment in both cases. The observation with scanning electron microscope showed a transition from cells with their standard morphology supported on clean filters to cells with a highly altered morphology lying on dirty filters with plenty of cellular debris. E. coli showed the most significant surface alteration. In conclusion, the results showed that E. coli is more resistant to electrochemical disinfection than B. subtilis and thus, it is a good surrogate. However, more studies with other different type of cells should be done to consider E. coli an adequate indicator of the electrochemical disinfection with BDD. Acknowledgements: We thank MINECO (Ministerio de Economía y Competitividad, Spain) for funding under project CTQ2013-48897-C2-1-R.

A potential role for cations in aquatic microbiology in urbanizing watersheds

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Catie Cappellin; Joshua Franklin; Megan Orentas; Regan Wagner; Meredith Steele

Increased salinity in developed watersheds results from changes in the concentration of different ions,
but the focus is often on total salinity. Understanding of the sources and relationships between specific cation concentrations and microbiology remains poorly developed. The objective of this study was to examine the relationship between a suite of water chemistry variables and microbial water quality indicators to identify indicators of surface water pollution and changing watershed processes. We collected weekly grab samples from nine sub-watersheds in southwest Virginia for a period of one year. Samples were measured for standard physical and chemical properties: dissolved oxygen, temperature, specific conductance, pH, calcium, magnesium, potassium, chloride, fluoride, sulfate, nitrogen species, phosphorus, and dissolved organic carbon. In addition, total coliforms, E. coli, and HF183 (a human-specific genetic marker, were measured as indicators of fecal contamination. Surprisingly, concentrations of traditional biogeochemical elements (N, P, C) were less strongly related to microbial water quality indicators than were Ca, Mg, Na in watersheds. Calcium and magnesium were strongly correlated with total coliforms, r² = 0.88 and r² = 0.86 respectively, while potassium was very strongly related to E. coli (r² = 0.96). Currently, we cannot reasonably explain these relationships by the land use composition or common sources within the landscape. The human-specific HF183 fecal indicator was not well correlated with other microbial water quality indicators, was found ubiquitously across the developed watersheds, and was most strongly correlated with sodium concentrations (r² = 0.84). The results suggest that 1) wastewater via subsurface flowpaths may more broadly impact surface water chemistry and quality than expected, and 2) that cation chemistry may influence the fate and transport of microorganisms in aquatic systems and serve as a background mediator of watershed biogeochemical cycling in cities.

**Fate and Transport of Fecal Indicator Bacteria (FIB) and Microbial Source-Tracking (MST) Targets in Groundwater**

Hannah Billian, Virginia Polytechnic Institute and State University

Leigh-Anne Krometis; Tess Thompson; Charles Hagedorn

Between 1970 and 2010 almost one-third of drinking water related waterborne disease outbreaks reported to the US Centers for Disease Control and Prevention were associated with systems dependent on untreated groundwater (i.e., most commonly, household wells). This is unsurprising, given that numerous past efforts to monitor household well water quality have indicated a high prevalence of fecal coliforms and/or E. coli at the point of use. Also, non-point sources of pollution, including septic tank leakages and poorly constructed drain fields, have been identified as the leading risk factors associated with outbreaks in households dependent on groundwater. Ideally, the integration of emerging MST analyses in well monitoring programs could be used to identify whether the presence of FIB is associated with human or non-human sources in order to inform remediation strategies. However, the application of MST to groundwater has been limited, and the interpretation of data is consequently of limited value. This research compares the fate and transport of FIB (E. coli and enterococci) with a chemical (optical brighteners, OB) and a molecular (Bacteroides HF183) source-tracking target in order to evaluate the potential use of such markers to identify water quality issues in private drinking water systems such as household wells. Eighteen PVC soil columns were constructed in an outdoor soil column facility to represent small-scale models of septic drainfields. The constructed columns received synchronized doses of primary -treated wastewater twice daily and were monitored over a seven-month period. Simulated events, including variable influent loading rates of wastewater...
effluent, and differing degrees of soil compromisation (channels), were carried out to monitor impacts on levels of source-tracking targets recovered from column effluents. Key impacts of this work include: relative recovery of MST and OB targets and FIB from controlled groundwater simulations to assist in the development of strategies to identify non-point sources of human wastewater pollution efficiently and effectively for remediation efforts.

**Microbe-Suspended Sediment Dynamics of Recreational Beach Water of the Chesapeake Bay**

Jon Calomiris, Sotiria Science

**BACKGROUND:** Microbe-sediment interactions of natural waters can present challenges to monitoring and maintaining the health quality of recreational beaches. Pathogens released into beach waters can sequester within sediment beds, survive for extended periods, and suspend into the water column by the actions of tidal currents, storms, bathers, and boaters. Suspended sediment can confound plate-count enumeration of fecal indicator bacteria (FIB) or pathogens since a single sediment particle harboring multiple organisms could yield one colony forming unit. In addition, sediment can interfere with molecular-based detection systems such as PCR. In this study, a recreational beach of a Chesapeake Bay tributary (Magothy River) was examined to understand the association of microorganisms with suspended sediment. **METHODS:** Samples of water with suspended sediment were centrifuged to collect single microbe-sized particles (MSP) in supernatants and pellet larger particulate masses. Single MSP and heterotrophic plate count (HPC) bacteria recovered in supernatants were enumerated by direct microscopic count and membrane filtration with R2A medium, respectively. Microbial attachment to suspended sediment was quantified on the basis of numbers of MSP and HPC bacteria released from particulate masses following dispersion with chemical and physical treatments. Dispersion of suspended sediment was observed by microscopic imaging (polarized light and bright field). **RESULTS:** The vast majority of HPC bacteria and MSP were associated with suspended sediment while relatively few resided in interstitial water. Combined chemical and physical treatments yielded the greatest dispersion of suspended sediment based on release of HPC bacteria and MSP. Microscopic examination revealed suspended sediment as floc-like masses harboring MSP with few free MSP. However, treatment disaggregated floc yielding small masses and many planktonic MSP. **CONCLUSIONS:** Disrupted estuarine sediment beds can release sediment particles sheltering large numbers of microorganisms into recreational waters. Beach waters tainted with suspended sediment could pose a health risk as well as challenge FIB or pathogen surveillance.

**Exploration of strategies to track the downstream impacts of human sewage in underserved rural communities**

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Leigh-Anne Krometis; Emily Sarver; Nicholas Cook

Monitoring pathogen contamination in receiving waters is critical for the protection of public health. Fecal contamination by human sources is generally considered a primary health concern as many waterborne pathogens (viruses in particular) exhibit host specificity with humans. Standard water quality assessments generally only include evaluation of total coliforms or E. coli, which are common to
all warm-blooded animals, and so provide limited information regarding specific upstream source remediation needs. The inclusion of library-independent, established source-specific markers in monitoring efforts can greatly aid in watershed assessment. The genetic marker HF183 from Bacteroides spp. has been well established as strongly indicative of the presence of human fecal contamination; however, to date, the majority of studies have documented HF183 detection downstream from relatively large centralized wastewater facilities, with usefulness in rural watersheds not well documented. The present study investigates water quality in small streams in central Appalachia known to have multiple discharges of untreated household waste (e.g. straight pipes). Efforts to document the extent of downstream impacts of these discharges included: 1) analysis of monthly water samples above and downstream wastewater discharge points via traditional methods for E. coli and molecular methods for HF183 to identify potential correlations in detection; and 2) completion of a geospatial statistical analysis to examine changes in microbial detection quantities based on season and distance from known contamination sources. Demonstration of successful use of molecular detection of HF183 in rural communities will provide water quality managers with a means to justify investment in local wastewater infrastructure.

Development of a One Health Sampling Scheme for Surveillance of Antimicrobial Resistant Bacteria in Northern Colorado

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Introduction: Antimicrobial resistant bacteria (ARB) are a growing global threat with increasing global attention, but coordinated international surveillance is lacking. Despite calls for worldwide harmonized approaches across diverse sample medias, persistence of differing methodologies across disciplines and lack of comprehensive, but equally-feasible, monitoring methods between developed and developing country sites hinder an effective global "One Health" ARB surveillance scheme. An interdisciplinary team from Fort Collins, CO, is collaborating with researchers in León, Nicaragua and Chapel Hill, NC in order to identify and validate a surveillance method that is versatile in its ability to be used across animal, human and environmental medias. Preliminary data for identification of multiple resistant bacteria using these accessible, affordable and practical methodologies for laboratories across the globe will be presented. Methods: A "One Health" sampling scheme was devised to include samples from wastewater treatment plant (WWTP) influent and effluent, hospital and community wastewater, hydrologically connected surface and recreational waters, livestock waste, and human samples. Samples are analyzed for the presence of Methicillinresistance Staphylococcus aureus (MRSA), Vancomycin-resistant Enterococcus (VRE) gram positive bacteria, and Extended Spectrum beta Lactamase Enterobacteriaceae (ESBL) and Carbapenem-resistant Enterobacteriaceae (KPC) resistant gram-negative bacteria. Enumeration is done for all E.coli and other total coliforms, Staphylococcus aureus and enterococci and of resistant MRSA, VRE, ESBL and KPC. Relative abundance and antimicrobial resistance properties are measured phenotypically by Kirby-Bauer disk diffusion susceptibility methods and by molecular characterization using multiplex PCR assays and random amplified polymorphism PCR. Mean recoveries are compared for equivalence using paired t-tests or non-parametric Mann-Whitney. Results: Based upon results from Nicaragua and North Carolina, we
developed a sampling scheme in collaboration with the City of Fort Collins, a local hospital, and local agricultural producers. The scheme presents a comprehensive "One Health" surveillance including: raw water sources from the Poudre River and Horsetooth Reservoir, Poudre River surface water sites upstream and downstream from community- and hospital-sewage processing WWTP, hospital and community sewage, WWTP plant influent and effluent, agricultural irrigation water sources, and dairy manure waste. Specific results from the enumeration and characterization of waste water treatment plant- and surface water-related samples will be presented. Conclusion: Results indicated applicability of methods used in North Carolina and Nicaragua to Northern Colorado, and specifically methods from human and animal medias to environmental samples. Clonality indicating emerging antimicrobial resistance shared across sample medias and global sites will subsequently be evaluated with isolates collected from this sampling scheme—with suggestion of wastewater as a source of hazardous ARB exposure. Results from Colorado based on method comparison, sample testing and enumeration from WWTP influent and effluent, discharge points, surface and recreational river waters, agricultural irrigation water, and livestock waste indicate feasibility of methods and presence of similar ARB hazard as seen in North Carolina and Nicaragua. Initial conclusions suggest exchange of ARB hazards between environmental wastewater, surface water, and agricultural water sites in Fort Collins as possible sources of human exposure and persistence of ARB in the environment and community. Funding Sources: CSU One Health and the Infectious Disease Supercluster

Characterizing rotavirus A from water samples in Nairobi, Kenya

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Group A rotaviruses (RVs) can cause severe and fatal diarrhea in children under five years of age worldwide. Rotavirus A (RVA) is predominantly transmitted fecal-oral, and has been detected in wastewater. In 2014, Kenya introduced live RV vaccines into the Expanded Programme of Immunization. Available vaccines include the RotaTeq® vaccine (G1, G2, G3, and G4 serotypes and P[8] genotype) and the ROTARIX® vaccine (G1 serotype and P[8] genotype). Since the introduction of RV immunization, no studies have characterized RVA strains from environmental samples. This study sought to characterize the RVA strains in circulation in the environment after the vaccine introduction. Water samples were collected from four sampling locations in Nairobi, Kenya: Kibera; Eastleigh A; Eastleigh B; and Starehe. One-hundred and eight (108) samples were collected from April 2015 to December 2015 using the recently developed bag-mediated filtration system (BMFS). Using the BMFS, 2.9 ± 0.4 L of the water samples was filtered through positively-charged ViroCap filters. Viruses were eluted from the filters followed by RNA extraction and RV detection by real-time RT-PCR. Nested RT-PCR typing of RVA was performed using G- and P-specific primers. Results for 31 samples collected from April 2015 to May 2015 are reported. Analysis by real-time RT-PCR showed all samples positive for RVA. Nested RT-PCR detected G1 (84%), G3 (71%), G8 (23%), and G9 (52%) serotypes. As the RotaTeq and ROTARIX vaccines do not include the G8 and G9 serotypes, these results correlate with those reported for clinical specimens and highlight the circulation of non-vaccine RV strains within these areas in Kenya, and could inform future vaccine development. Also, P[8] genotype was detected in all samples suggesting that P[8] could be used as a recovery control. This method does not distinguish between wild type and vaccine strains. Therefore, future studies should use more specific
probes to differentiate wild type and vaccine strains.

**Water Quality and Fecal-Indicator Detection in Response to an Impaired Urban Watershed, Turkey Creek: A "Gulf of Mexico Initiative Focus" and a "Making a Visible Difference" Program**

Stephanie Friedman, EPA/ORD/NHEERL/GED

Jerry Boos; Lael Butler; Katelyn Houghton; David Beddick; Tripp Boone; James Farmer; Judy Steckler; Paulette Carter; Troy Pierce

The historical communities of Turkey Creek originated in 1866, when a group of emancipated African-Americans purchased land in Harrison County, MS, along the Turkey Creek watershed. Many of the current members of this community are descendants from the original settlers. This watershed provided a way-of-life for the settlers and for the present-day communities including fishing, recreation and community baptisms. What was once a forested and riparian floodplain has been dramatically altered as the influx of commercial development, an international airport and surfaced roadways merged the Turkey Creek community into the city of Gulfport, MS. EPA is focused on lending support to local communities that are environmentally overburdened, underserved, and economically distressed as part of the Agency's "Making a Visible Difference" program. The Turkey Creek watershed is listed as a Total Maximum Daily Load (TMDL) impaired water body for fecal coliforms on the Mississippi 1998 Section 303(d) list and in the January TMDL 2015 report and has become a high-priority watershed on the "Making a Visible Difference" (MVD) program for the Agency. In response to their needs, EPA will help the community align environmental concerns with economic priorities, including community involvement, community organizations, and connecting the community to local officials. The EPA Gulf of Mexico Program initiated a Citizens' Science partnership to increase water-quality monitoring and community participation within the Turkey Creek watershed. Students from a near-by community college and middle school, along with their EPA partners, are collecting water samples at various previously-determined impaired stations along the watershed and using the IDEXX method to quantify weekly E. coli levels. Stations with higher levels of E. coli will be studied more in-depth to evaluate fecal contamination source. The monitoring data collected by the students and EPA staff are regularly presented to the Turkey Creek Steering Committee and in the future, the source-tracking results should provide additional leverage to support decisions and target environmentally-feasible solutions. This presentation will show-case the Citizens' Science project along with the environmental data.

**Exposure Assessment of Livestock Carcass Management Options for Natural Disasters**

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Kaedra Jones; Margaret McVey; Tao Hong; Natalie Blanton; Sarah C. Taft; Paul M. Lemieux; Lori P. Miller

Management of livestock carcasses following large-scale mortalities is needed to protect humans, livestock, and wildlife from hazards; to maintain water, air, and soil resources; to protect ecological resources; and to enhance food and agricultural security. Previous health and environmental
Accelerating the process of solar disinfection (SODIS) by using polymer bags

Sergio Gutiérrez, University of Cadiz
Asunción Acevedo; Manuel Figueredo; Matthias Saladin; Manuel Manzano

Background The method of solar water disinfection (SODIS), consisting of filling common plastic bottles with water and exposing them to the sunlight for at least six hours, is among the available solutions to treat the water at the point-of-use to inactivate different types of pathogens. The aim of this project was to investigate and develop a SODIS bag meeting technical requirements (effective disinfection, safe storage, high temperature) and practical use (easy to fill, hang, drain, store and transport), and to evaluate its use and acceptance in developing countries. In this work, several bags made of different polymers were tested in laboratory conditions. The two best were subjected to continuous use in simulated field conditions. Finally, the best prototype of SODIS bag was tested in Haiti in cooperation with the NGO CARE. Methods Experiments using bags of different materials were carried out under real sunlight with mineral water inoculated with a known strain of E. coli. In a second stage, two improved design prototypes (tap, wide opening, 4 liters capacity), made of the previously selected materials (PE and PE/EVA), were subjected to disinfection tests of wild strains of E. coli and fecal enterococci in contaminated groundwater. Finally, a field study was implemented in Haiti, in cooperation with the NGO CARE Haiti. For this purpose, 800 bags of the best prototype were distributed among 200 families which had been previously trained in the use of SODIS method. Results and discussion Taking into account sunlight transmittance spectra, preliminary disinfection results and
mechanical properties, the materials selected from the preliminary tests were PE and PE/EVA bags (and PET bottle for comparison). The bags with higher UV-B transmittance reported faster disinfection. The PE bag achieved most rapid disinfection of wild E. coli and Enterococcus spp., followed by PE/EVA bag; least was the PET bottle. After 5 months of continuous use, disinfection effectiveness was similar for both prototype bags. Nevertheless, PE bags suffered more frequent breakages, having a shorter lifespan than PE/EVA ones, which lasted more than 6 months without loss of efficiency and without breakage. Therefore, PE/EVA bag was chosen for field trials in Haiti. After 3 months of using SODIS bags and PET bottles, field surveys reported that the majority of users preferred the SODIS bag, due to its easy handling, higher volume or better taste of treated water. A few people described a plastic taste that disappeared after a few uses. Compared with other methods of point-of-use water disinfection, most of the users preferred the SODIS method, especially compared to chlorination, which imparts strong taste to the water and was perceived as "artificial" or "chemical" against the "naturalness" of using sunlight. Conclusions In summary, replacing bottles with a suitable bag not only reduces the space occupied while transporting, but also improves the efficiency of SODIS treatment and facilitates its use, improving user acceptance and thereby increasing success in promoting the SODIS method. The SODIS bag shows high potential for application at household level or in emergency situations where transport and logistics are challenging.

Correlating the relative abundance of ammonia and nitrite oxidizing bacteria with the nitrification performance using Hierarchical Oligonucleotide Primer Extension (HOPE)

Peiying Hong, King Abdullah University of Science and Technology, KAUST

GianTommaso Scarascia; Hong Cheng

The conventional process of removing nitrogen from municipal wastewater streams involves nitrification followed by denitrification. Nitrification in the wastewater treatment process is generally performed in two consecutive steps by ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). Establishing an optimal ratio of AOB to NOB is hence important in ensuring the functionality of the nitrification process. A high-throughput method to quantify for the abundance of the AOB and NOB would be particularly useful in tracking the proportional ratio between these two groups that are important for the nitrification process. In this study, we develop the hierarchical oligonucleotide primer extension (HOPE) approach to target the predominant groups of AOB and NOB in the wastewater environment. The method was tested against samples collected from a lab-scale biofilm-based trickling bioreactor. The bioreactor was set up to enrich for AOB and NOB, and the reactor was challenged to high concentrations of incoming ammonium content so as to simulate a toxic shock event that could potentially crash the nitrification system. The change in AOB and NOB proportions was tracked by the HOPE approach, and correlated to the reactor performance. The total microbial community was also elucidated by the 16S rRNA gene-based next generation sequencing. Our findings indicate a significant increase in the abundance of AOB with increasing ammonium content (t-test, p < 0.001). In contrast to the Nitrosomonas, Nitrosospira detected by primer NspCL1 and Nsv443 remained stable in its relative abundance against the total community throughout the operational phases but were in lower relative abundance compared to the Nitrosomonas. There was a corresponding significant decrease in the relative abundance of NOB group targeted by Ntspa572 primer (p = 1.1 x 10^-4). Although Nitrobacter detected by Nit1017 primer increased in its relative abundance from 4.8
± 3.2% in early operational phase to 9.0 ± 4.6% in the late phase, there was no corresponding decrease in the nitrite content, suggesting that Nitrobacter may not be playing as important functional role as Nitrosospira. When compared to the reactor performance evaluated based on the water quality, it was determined that the ratio of AOB against both NOB groups ranged from 0.7 to 2.5 during the phases that correspond to stable reactor performance. During phases that correspond to a deterioration in the reactor performance, the ratio of AOB against Ntspa572-targeted NOB however increased to a range of 11.7 to 13.4. The ratio of AOB against Nit1017-targeted NOB also increased to a range of 2.9 to 3.6. The multivariate mMDS plots from both HOPE and next generation sequencing datasets were compared and showed good correlation at a significant confidence level (p = 0.79, p = 0.001). This study demonstrates the development of HOPE as a high-throughput method to quantify for the predominant AOB and NOB groups that have been identified till date in conventional wastewater treatment systems. The method allows for the simultaneous monitoring of relative abundance of AOB and NOB groups, which would be indicative of the reactor performance and nitrification functionality.

**Antibiotic Resistance Characterization of Surface Water Bacteria in an Urbanizing Watershed**

Dylan Laird, Texas A&M University

Terry Gentry; John Brooks

Wastewater treatment plants (WWTP) may place selective pressure on exposed bacteria for the proliferation of antibiotic (AB)-resistant traits that could then be introduced into the environment. To characterize the resistance of surface water bacteria in an urban stream setting, E. coli isolates and total heterotrophic bacteria (HPC) were cultured from 6 sites in the Carter Creek watershed of College Station, TX, and evaluated for resistance to select ABs. HPC were cultured on R2A amended with selected ABs, and compared to culturable populations on AB-free media. E. coli were isolated using EPA Method 1603 and tested for resistance to 8 Abs (ampicillin, tetracycline, sulfamethoxazole, ciprofloxacin, cefalothin, cefoperazone, gentamycin, and imipenem) using the Kirby-Bauer method. HPC counts produced a wide variation in the ratio of viable cultures between control and amended media, likely due to variability in the watershed. All E. coli isolates displayed susceptibility to imipenem; however, the majority (85%) showed resistance to cefalothin, and lesser resistance to each remaining AB tested. A substantial fraction (21%) of E. coli isolates were resistant to two or more antibiotics, with 63% obtained downstream of a WWTP. Isolates expressing resistance to three or more antibiotics comprised 10% of the total isolates tested. During three of four sampling events, isolates expressing resistance to three or more antibiotics were limited to only sites downstream of a WWTP. The majority of multidrug-resistant isolates that were obtained upstream of a WWTP were collected during a single sampling event, while multidrug-resistant isolates obtained downstream of WWTPs were spread more evenly across all sampling events. Initial results suggest the possibility of a variable resistance profile moving downstream through the watershed, and may indicate an influence of urbanization on bacterial resistance characteristics expressed in the surrounding environment.

**Environmental Surveillance of Polio in Kenya: Key Lessons Learned**

Jeffry Shirai, University of Washington
International collaborative studies on environmental surveillance are challenging to conduct. Sample preservation, shipping logistics, permitting, and laboratory capacity and resources must be coordinated. An ongoing polio surveillance project funded by the Paul Allen Family Foundation has brought together partners at the University of Washington (UW), Kenya Medical Research Institute (KEMRI), University of Pretoria (UP), PATH, World Health Organization (WHO), Centers for Disease Control and Prevention (CDC), and the Bill & Melinda Gates Foundation. This multi-year study is intended to compare environmental surveillance for poliovirus using the WHO’s grab sample/two phase PEG-Dextran separation method to a novel new method using a bag-mediated filtration system (BMFS) developed at the UW. In an effort to work out logistical challenges prior to full validation, a pre-validation study was conducted between April and September 2015. BMFS samples were collected alongside WHO grab samples as part of the routine environmental surveillance program in Kenya. Samples were collected at four sites in Nairobi (Starehe, Kibera, and two Kamukunji locations), twice every month for a total of almost 60 samples. All filter samples were shipped to the UP for processing and laboratory analysis. Although the basic study design appears straightforward, numerous logistical, technical, and safety considerations had to be addressed during the preparation for and implementation of the pre-validation study. Not overburdening the routine sampling activities and consideration of traffic were important factors on the sampling side, whilst the timing of the sampling had to be coordinated to facilitate timely shipping and receipt of the samples by the processing laboratory. Sample integrity and preservation were of primary concern and came into play during the transport of wastewater sample collection from the field to the KEMRI Laboratory and during courier service from Nairobi to Pretoria. Customs delays in partner countries were an ever-present worry. Preservatives (2% sodium benzoate and 0.2% calcium propionate), cold-chain techniques, and the introduction of an MS2 tracer were employed to extend sample holding times. Real-world issues were in stark display as one sampling site had security-related concerns caused by individuals associated with a nearby illegal distillery while another site attracted large crowds that were difficult to control and made for an uneasy situation for the field sampling team. Administrative hurdles including the iterative review of multi-organizational contractual agreements, procurement of in-country study permits, and attainment of import/export permits all proved to be months-long processes. Feedback from field sampling partners was invaluable in overcoming these issues, as it provided suggestions of how various aspects of the sampling kit and protocol could be modified. Examples included the development of a bucketing protocol that allowed field samplers to transport water samples to safer or central locations for processing, and the introduction of a reusable housing to minimize waste. This presentation will discuss the many lessons learned in addressing these challenges associated with project coordination, and sample collection, transport, and analysis in multiple countries. These insights can readily be utilized in other multi-national studies that involve water or wastewater sampling.

**Patterns of antimicrobial resistance in E.coli isolates from soil and water samples in three Texas watersheds with different land uses.**

Maitreyee Mukherjee, Texas A&M University
Antibiotics are used globally to protect human and animal health, and also as feed additives to augment the growth rate of animals. The widespread overuse of antimicrobial agents has led to the pressing global problem of the occurrence and spread of antibiotic-resistant bacteria, leading to the emergence of multidrug resistance, that is increasingly compromising the treatment of life-threatening diseases globally. Although many studies have focused on determining the patterns of antibiotic resistance from domestic animals and farm environments, very little is known about environmental and natural reservoirs of antibiotic and multidrug resistance and the impact of agriculture on this. In this study, we collected stormwater runoff using automated samplers and soil samples (5 cm depth) from three different watersheds in Texas with varying land uses - managed hay pasture, native prairie, and cropland. E.coli was isolated and enumerated from each of these sites using the EPA method 1603. The isolates were analyzed for patterns of antibiotic resistance by antimicrobial sensitivity testing using the Kirby-Bauer disk-diffusion method for multiple antibiotics: tetracycline, imipenem, cephalothin, gentamycin, sulfamethoxazole, cefoperazone and ampicillin. In general, a higher rate of antibiotic resistance was observed within the water E.coli isolates (63%) compared to the soil isolates (40%). Overall, more isolates were resistant to the cephalothin - than any of the other antibiotics - 40% and 52% of soil and water isolates, respectively. Of the tested E.coli isolates from the water samples, 63% were resistant to one or more antibiotics, with 52% being resistant to cephalothin, 26% resistant to tetracycline, and 10% resistant to ampicillin. Furthermore, 21% of the water isolates demonstrated multidrug resistance (resistant to 2 or more antibiotics). All of the soil isolates exhibiting antibiotic resistance were resistant to the cephalothin (40%), but only three of these isolates were multidrug-resistant (2.5%). The pattern of high cephalothin resistance observed within both the soil and water isolates may suggest the presence of native populations of cephalothin-resistant bacteria within these sites. However, the higher rate of multidrug resistance (~10 times higher) observed within the water isolates when compared to the soil E. coli populations suggests the possibility that resistance sources other than just the soil contributed to antibiotic-resistant E. coli in runoff from these watersheds.

**Actinobacteria isolated from deep-water endemic amphipods of Lake Baikal as a source of novel biological active compounds**

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Denis Axenov-Gribanov; Irina Voytsekhovskaya; Maria Dimova; Ekaterina Madyarova; Kseniya Vereshchagina; Renat Adelshin; Maxim Timofeyev

Actinobacteria are one of the most prominent source of biological active compounds (Berdy 2005). Since the 1940s soil was the main place to isolate new strains of actinobacteria. Recently many studies devoted to investigation of extreme ecosystem to obtain novel strains and isolate new biological active metabolites (Karuppiah et al. 2015; Fang et al. 2015). One of this ecosystems is Lake Baikal. It is the largest lake by volume in the world containing roughly 20% of fresh water. Lake Baikal is the deepest and oldest lake of the world with a maximum depth of 1.642 m and highly stable environmental condition. Lake’s water is well-oxygenated (Timoshkin et al. 2001). These and others factors make Baikal ecosystem unique. In these unique conditions there are numerous representatives of Baikal endemic extremophile fauna evolved last millions years. One of the dominant group of Baikal endemic
organisms, which evolved in the stable conditions, is Amphipods (Crustacea). Deep-water endemic amphipods Ommatogammarus albinus and O. flavus are benthic scavengers (Tahteev 2000). We hypothesized that living near the bottom, deep-water amphipods can be associated with unknown actinobacteria. Using traps with sterile putrescent fish we collected deep-water amphipods belongs to genus Ommatogammarus (O. flavus and O. albinus) from the depth 80 - 200 m. From the samples it has been obtained 43 pure cultures of actinobacteria with using selective media (MS, SG, ISP, Gauze's synthetic agar, and Wakman media) and heat-shock pretreatment. Most of the strains belong to the genus Streptomyces but also it was found specimens of the Micromonospora and Pseudonocardia genera. It has been provided antibiotic activity tests in order to estimate the biologically active compounds of isolated strains. The strains were grown in two variants of liquid media such as NL-19 and SG. The crude extracts from culture liquid and biomass were used for estimation of activity against a number of test microorganisms - Bacillus subtilis ATCC 6633, Pseudomonas putida KT2440, Staphylococcus carnosus ATCC 51365, Escherichia coli ATCC25922, antibiotic resistant E. coli K12 (resistant to ampicillin and kanamycin), Saccharomyces cerevisiae BY4742 and Candida albicans ATCC 90027. Most of the isolates were obtained from the depth 200 m. Thus 25 isolates were obtained from O. flavus and one from O. albinus. From the depth 80 m it was obtained 11 strains from O. albinus and 4 from O. flavus. From the depth 100 m two isolates were obtained from O. flavus only. Most of the strains did not reveal the activity against Gram-positive bacteria B. subtilis and St. carnosus (39 out of 43). Only a few strains (Streptomyces IB 2015 /P / 113-1, Streptomyces IB 2015 /P / 113-2, Streptomyces IB 2015 /P / 113-12, Streptomyces IB 2015 /P / 119-5 grown in NL-19 media and Streptomyces IB 2015 /P / 113-12 grown in SG media) showed activity against Gram-negative microorganisms P. putida and E. coli. 16 strains showed antagonistic activity against C. albicans and some of them also were able to inhibit the growth of S. cerevisiae. In conclusion actinobacteria isolated from deep-water amphipods of Lake Baikal are promising source of novel biologically active compounds. Some of the obtained strains revealed wide range of inhibiting test cultures while others showed specificity against certain type of microorganisms. This study was partially supported by the Ministry of education and science of Russian Federation as a part of Goszadanie projects (№6.382.2014/K, 6.734.2016 DAAD, 6.696.2016 DAAD), Russian science foundation (project N 14-14-00400), Russian foundation for basic research (projects N 14-04-00501, 15-54-04062, 16-34-0068), Grants of Irkutsk State University for researchers. List of references Berdy, János. 2005. "Bioactive Microbial Metabolites." The Journal of Antibiotics 58 (1): 1-26. doi:10.1038/ja.2005.1. Fang, Baozhu, Chongxi Liu, Xuejiao Guan, Jia Song, Junwei Zhao, Hui Liu, Chuang Li, Wenxi Ning, Xiangjing Wang, and Wensheng Xiang. 2015. "Two New Species of the Genus Micromonospora : Micromonospora Palomenae Sp. Nov . and Micromonospora." Antonie van Leeuwenhoek 108 (1). Springer International Publishing: 141-50. doi:10.1007/s10482-015-0472-9. Karuppiah, Valliappan, Yingxin Li, Wei Sun, Guofang Feng, and Zhiyong Li. 2015. "Functional Gene-Based Discovery of Phenazines from the Actinobacteria Associated with Marine Sponges in the South China Sea." doi:10.1007/s00253-015-6547-8. Tahteev, VV. 2000. Essays on the Amphipods of Lake Baikal: Systematics, Comparative Ecology, Evolution. Irkutsk: Irkutsk State University Press. Timoshkin, O.A., T.Y. Sitnikova, O.T. Rusinek, N.M. Pronin, V.I. Proviz, N.G. Melnik, R.M. Kamaltynov, D.F. Mazepova, and A.V. Shoshinin. 2001. Index of Animal Species Inhabiting Lake Baikal and Its Catchment Area, Vol. 1. Novosibirsk: Nauka.

Microbiological and physico-chemical characteristics of surface water collected from Tiaoxi river (Taihu watershed), China
Lake Taihu is the third largest freshwater lake in China, located in the Chanjiang Delta of the Yangtze River and serving as drinking water source for approximately 30 million residents. This lake is connected with several rivers, and Tiaoxi river is one of the main contributors to Taihu lakes water input. Previous reports showed that Taihu lake is suffering from water quality issues such as eutrophication, cyanobacterial blooms and fecal pollution due to various discharges in to the watershed. Therefore, the main aim of this study was to assess the microbiological and physico-chemical characteristics of the Tiaoxi river. The water samples were collected from 25 sampling locations along both East and West Tiaoxi river up to the Taihu lake junction. The sampling was performed in autumn 2014, summer 2015 and winter 2015 to address seasonal variation. The sampling locations were selected on the basis of importance and land use pattern. Physico-chemical (e.g. multiple nutrients), microbiological (e.g. total viable count, E.coli/Total Coliform and Fecal coliform) and molecular (e.g. 16S rRNA gene copy numbers) analyzes were carried out. The values of many physico-chemical parameters were within the recommended World Health Organization guidelines for surface water, however, the total phosphorous (TP), nitrate-N and total organic carbon levels were higher than the recommended values in several locations, suggesting possible contamination with domestic waste and/or agricultural run-off into the river. Notably, the fecal coliform (FC) counts were significantly higher than the acceptable range in 15 sampling locations and the highest count was observed in sampling locations close to the area where people live on boats without proper sanitation facilities. The total coliform and heterotrophic plate counts were also higher in those sampling locations. Significant seasonal variation was observed with parameters such as TP, ammonia-N, TOC and fecal coliforms. Results from the microbiological analyses indicate possible fecal contamination of water in several locations along the river. In order to study the sources of fecal pollution, a microbial source tracking study is underway in order to ascertain the identity of the possible sources contributing to this fecal pollution in Taihu watershed.

Comparison of the qPCR and the HybriScan Legionella assays vs. culture for the identification of Legionella sp. in non-potable water samples

Rosemarie Read, NorthEast Ohio Regional Sewer District

Chris Lannan; Mark Citriglia

Legionnaire's disease is a severe form of pneumonia caused by Legionella sp., with the majority of cases due to Legionella pneumophila. Mode of transmission includes the inhalation of contaminated, aerosolized water droplets from man-made water sources such as cooling towers, and aspiration of contaminated liquids. Outbreaks and sporadic cases of Legionellosis within communities have been attributed to contaminated, aerosolized water stemming from industrial buildings in close proximity to these communities. In order to evaluate and control the levels of Legionella sp. from these sources, constant monitoring of bacterial levels is recommended. Currently, the gold standard for identification and monitoring of Legionella sp. is by culturing samples onto selective and enriched media. However, this method for Legionella sp. identification has its drawbacks, including long incubation time (up to 10
days), inability to grow viable but non-culturable cells, and the difficulty in confirming serogroup or species. Therefore, it would be of benefit to apply methods for the detection of Legionella sp., in addition to the culture method, that will provide results earlier, and verify the identification of the organism. Quantitative PCR and the HybriScan Legionella assay are rapid methods for detecting Legionella sp. in water samples. Results from these assays can be obtained within the same day of processing. The qPCR method detects total Legionella DNA from the sample, but does not differentiate DNA from live vs. dead cells. The HybriScan assay detects Legionella rRNA by sandwich hybridization. Because rRNA is quickly decomposed in dead cells, the HybriScan assay detects only living cells. Both these methods are currently being employed, in addition to the culture method, by the Analytical Services department of the Northeast Ohio Regional Sewer District on boiler system samples obtained from the wastewater treatment plants. Results from these comparison studies, and their advantages, will be assessed.

**Opportunistic Pathogens in a Hot Water Pipe Loop System**

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Opportunistic pathogens found in drinking water, including Mycobacterium, Legionella, and Pseudomonas species, are a growing public health concern because of their increasing frequency as causes of infection. In the United States, non-tuberculous mycobacteria (NTM) prevalence increased 8.2% from 1997 to 2007 [1], while the prevalence of Legionella infections increased by 192% from 2000 to 2009 [2]. The presence of these pathogens in drinking water is particularly problematic for sensitive individuals, including the elderly, young, and immunodeficient, who are at greater risk of severe and fatal illness from exposure. Drinking water distribution systems (DWDS) are a possible harbor for opportunistic bacteria. Although drinking water treatment plants in the U.S. provide a disinfectant residual to prevent microorganism regrowth in the DWDS, the chemical, physical, and biological parameters of complex pipe networks can shelter opportunistic pathogens and allow for their growth. These factors affect the drinking water microbiome. In particular, biofilms and intracellular growth in protozoa can protect bacterial pathogens from adverse conditions in the DWDS. Furthermore, water quality parameters, such as temperature, metals, nutrient concentrations, and disinfectant type, can correlate with the growth of certain microorganisms in the DWDS [3]. For instance, Legionella thrives in hot water at temperatures up to 60° C [4]. Increased microbial growth in hot water pipes is a concern in large buildings and hot water loops, where warm water remains in pipes for longer durations before reaching the consumer. These types of systems are often found in hospitals and residence homes that provide hot water to high-risk sensitive populations. Compounding the effect of large hot water systems, water in hot loops is often recirculated, allowing pathogens to remain in the system [3]. In this poster presentation, we describe our research on a hot water pipe loop that is fed by municipal drinking water. The water is heated and then supplied through a pipe loop to a university campus and nearby hospital buildings. Unused water is recirculated in the pipe loop. During multiple sampling events at locations within the hot pipe loop, water was collected and analyzed for microbial and chemical water quality parameters. Chemical attributes of the system, including temperature, pH, total and free chlorine, and total and dissolved metals, were measured for each sample. Biological analyses included total bacterial counts by fluorescence microscopy and qPCR-based analyses to
Rapid and efficient extraction method of chlorophyll by bead beating

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Estimation of chlorophyll a has been routinely applied for determination of algal and phytoplankton levels in marine and freshwater to assess the eutrophic status and thereby monitor water quality. The existing method of extracts chlorophyll a from glass fiber filters by manual mastication in presence of cold 90% acetone. The manual mastication of the filters is a prolonged and arduous process that allows processing of only two samples (in triplicates) in 20 minutes i.e. about 3 minutes on average for processing each glass fiber filter. An alternative method of bead beating the glass fiber filters using an Omni bead ruptor 24. The new method significantly reduced the time of processing of two samples analyzed in triplicates. Comparative analyses of the new extraction method and the existing method were done keeping all other processing parameters constant and the chlorophyll a content was determined. Comparison of the results from both the methods were statistically analyzed by Passing and Bablok regression which suggests that there were no significant systematic and no proportional differences between the two methods of extraction. It was also noted that the standard deviations amongst the data obtained by the bead beating method were less compared to the existing method, which probably is due to less handling required. We conclude that the application of bead beating of the glass fiber filters can be used with better efficiency to routinely process chlorophyll samples.
Meta-analysis of seasonal concentration and flow-based results for Escherichia coli and Bacteroidales for inland recreational waters from middle Tennessee

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Fecal indicator bacteria, such as Escherichia coli (E. coli), are frequently monitored in recreational waterbodies as indicators of potential fecal pathogen presence and exposure. Members of Order Bacteroidales are considered alternative indicators that show usefulness in more quickly establishing fecal contamination in waterbodies by molecular techniques. A meta-analysis was performed on a database containing samples collected for a National Pollutant Discharge Elimination (NPDES) stormwater permit from inland recreational waters in central Tennessee (Nashville), USA during 2007-2012. The objective of the analysis was to compare seasonal concentrations and loadings, two common ways to measure water quality, for the individual fecal bacteria. All samples were collected during baseflow stream conditions (<0.1" rainfall within last 72 h) and a subset of these samples had simultaneous velocity readings taken by a Swoffer Model 3000 Current Velocity-Flowmeter. Samples were analyzed for E. coli using the Colilert method. The number of Bacteroidales 16S rRNA gene copies was determined by the AllBac real-time quantitative PCR assay without DNA extraction. Results were reported in both concentration (units/volume) and loadings (units/time). Highest concentrations and loadings occurred in the summer and spring, respectively, for both fecal bacteria. Lowest concentrations and loadings were found in the winter and fall. This study highlights the need for more research aimed at investigating the influence of factors on fecal indicators after they are deposited into a waterbody.

Detection of waterborne enteropathogens from river water sample to trace the source of contamination

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Background: Kathmandu Valley, the capital city of Nepal is drained by Baghmati river basin which caters the daily household need of water supply for the residents of valley. In this study, water samples from Baghmati river were collected and analyzed for the detection of water borne enteropathogens including bacteria, protozoa and virus in order to study the dynamics of microbial population as well as the contamination intensity of river water as it entered through the area of its origin to the core settlement region of the city and flowed all the way down to the region of least population density of Kathmandu Valley. Methodology: A total of twelve water samples were collected periodically (monthly) for four months (October, 21015 to January, 2015) from three different spots of Baghmati river basin. The sampling spots selected comprised of the upstream region of the river (Sundarijal), the midway region as the river passed through the settlement area of highest population density (Thapathali) and the downstream region of least population density (Chovar) as the river flows past the town. Indicator bacteria for fecal contamination especially total coliform and E. coli were detected by using Colilert Reagent and other pathogenic bacteria were detected by conventional culture and

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biochemical test. Similarly, protozoa were detected by microscopy and modified acid-fast staining was
done for identification of coccidian parasites. Meanwhile, for rapid screening of diarrhoeagenic virus
(special interest in detection of Rotavirus antigen), Enzyme Immuno assay was carried out. Results: We
found the highest intensity of fecal contamination in samples collected from Chovar for all four months
followed by Thapathali with average value ranging from 2.1X106 CFU/ml for total coliform count and
3.2X106 CFU/ml for total E. coli count. During the four months study period, the highest number of
coliform and E. coli was detected in the month of November 2 X106 CFU/ml and 4 X106 CFU/ml
respectively. Beside these other bacterial pathogens i.e. Pseudomonas spp., Klebsiella oxytoca,
Acinetobacter spp., Salmonella spp., Vibrio spp., were detected. Similarly parasites recovered were
Entamoeba histolytica, Entamoeba coli and Cyclospora spp. Rotavirus was detected from the water
sample collected from Thapathali in month of December 2015. Conclusion: Aberrant yet intriguing; the
result of our study demonstrated higher fecal contamination in the downstream region of the river, an
area of the least population density rather than midway region where the river passed through high
population density settlement. This may be due to the direct discharge of sewage, garbage and other
animal faces in the river from the industries which are located around Chovar. Further research, such
as Microbial Source Tracking (MST), will be conducted to identify the origin of fecal contamination.

Non-tuberculous Mycobacteria in Point-of-Use Water Sources in Homes: A Case-Control Study of
Association with Human Disease

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Maegan Dirac; Annie Becker; Nicola Beck; Kris Weigel; J. Scott Meschke; Gerard Cangelosi

Disease exposure in the home is a growing concern in public health. While many opportunistic
pathogens have been identified in point-of-use (POU) water sources in homes, it can be difficult to
correlate these observations with human health outcomes. Infectious diseases such as Mycobacterium
avium complex pulmonary disease (MAC-PD) are commonly associated with host-specific disease risk
factors, but environmental exposure factors are also likely important. Here we report a secondary
analysis of a case-control study with 70 MAC-PD cases and 61 age-, gender-, and geographically-
matched community controls in Washington and Oregon. We analyzed the colonization of case and
control homes by non-tuberculous mycobacteria (NTM), including MAC, and assessed the correlation
between colonization and MAC-PD. Mycobacteria were cultured from home environmental samples
collected at five POU sites, namely indoor soil, outdoor soil, bathroom shower aerosols, bathroom sink
faucets, and kitchen sink faucets. Colony-purified isolates were categorized by PCR reactions including
a genus-specific Mycobacterium PCR as well as species-specific reactions targeting Mycobacterium
intracellulare, Mycobacterium avium, and Mycobacterium avium-intracellulare. Case homes were
more likely to yield positive NTM and/or MAC isolates than control homes considering all five POU sites
as a total home exposure (p = 0.040). Total water-associated point-of-use sites in case homes
(bathroom shower aerosols, bathroom sink faucets, and kitchen sink faucets) also showed higher
positive counts (p = 0.047). Indoor and outdoor soil sites did not exhibit this correlation. These findings
suggest that certain point-of-use environments in case homes may be more favorable to mycobacterial
colonization than those of control homes. To our knowledge this is the first case-control study to
correlate mycobacterial colonization of POU water sources with human disease.
Comparative decay kinetics of fecal indicator bacteria, host-associated fecal markers, and pathogens in a coastal California lagoon

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Jared Ervin; Patricia Holden

Coastal waters are frequently contaminated with elevated concentrations of fecal indicator bacteria (FIB) that may signal a risk to human health. However, FIB concentrations may be elevated by other than human sources of fecal pollution. To better assess microbiological threats to public health, host-associated fecal markers via qPCR are used in microbial source tracking (MST) to determine if human and other sources of fecal contamination are present. However, the relationships between host-associated fecal marker, FIB, and pathogen concentrations are not well understood in environmental waters. Coastal lagoons in many Southern California watersheds form an important intermediary between runoff from urbanized areas and popular beaches. Lagoons may be zones of FIB attenuation or amplification, with seasonally-dependent hydrology affecting the dominant process (Steets et al., 2003). However, little is known regarding host-associated marker fates in coastal lagoons, including their persistence or decay patterns relative to FIB. To better understand how lagoons may affect downstream water quality and microbiological risks to beachgoers, marker fates in lagoons should be known. The Arroyo Burro watershed in Santa Barbara, CA exemplifies a suburban coastal watershed with an intermittently discharging coastal lagoon. Dialysis chambers containing diluted sewage were deployed in the lagoon and sampled daily for ten days under summer (sunlight vs. shade) and winter conditions. Samples were analyzed for culturable FIB (E.coli, enterococci), host-associated fecal markers for human (HF183, HumM2, BacHum) and dog (DogBact), general fecal markers (Entero1A, GenBac3), bacterial pathogens (Campylobacter), and viruses (human adenovirus). Results showed differential decay rates between culture-based FIB, host-associated fecal markers, and general fecal markers. Except for E. coli, decay rate constants were similar in sun or shade during the summer; rate constants in the summer exceeded those in winter for most other assays. Wintertime rate constants were similar between FIB and the host-associated and general markers, yet there were marked differences in summer. In particular, human and dog host-associated fecal markers decayed at faster rates than culture-based FIB, general fecal markers, and pathogens. Regardless of season, Entero1A was the most persistent marker and displayed a slower decay rate than the culture-based enterococci. These results suggest that in a brackish lagoon environment, host-associated markers may be used as indicators of recent pollution during the summer. However, the use of these markers as an alternative indicator to human health risks may be limited due to their more rapid decay compared to the pathogens evaluated in this study. Steets, B. and Holden, P.A. 2003. A mechanistic model of runoff-associated fecal coliform fate and transport through a coastal lagoon. Wat. Res. 37:589-608

Optimal strategies for monitoring irrigation water quality and the development of guidelines for the irrigation of food crops

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Kelly Bright; Kelly Reynolds; Nathan Lothrop; Jonathan Sexton; Channah Rock
Historically, water quality guidelines have focused on drinking, waste, and recreational sectors, excluding waters used throughout the production of food crops. The agriculture industry has recently developed produce safety guidelines aimed at minimizing contamination throughout the growing, packing, shipping, and processing operations (Food and Drug Administration, 2015). Although these guidelines (Standards for Growing, Harvesting, Packing, and Holding Produce for Human Consumption) aim to establish science-based standards for agricultural processes, they fail to grasp the complexity of irrigation systems and offer few suggestions for appropriate monitoring of irrigation water safety. Therefore, better characterization of the microbial quality of irrigation water systems was needed to reduce risk at the point of irrigation, water extraction, and application. The overall goal of the proposed study is to assess the spatial and temporal occurrence of microorganisms in irrigation water systems to determine the most appropriate sampling strategies that aid in risk reduction practices for agricultural water used for food crops. Four specific objectives aimed to accomplish this goal: 1) determine the appropriate time of day for irrigation water monitoring; 2) determine the appropriate sample collection point across canal transects; 3) address the transport of microorganisms in irrigation canals; 4) determine if it is more appropriate to collect a single sample, multiple samples, or composite samples. Finally, a fifth objective defined an overall sampling strategy to produce the most relevant data for determining the risks of microbial pathogen contamination of food crops via E. coli contaminated irrigation waters. Water grab samples (n=1,367) were collected between December 2014- November 2015 from irrigation canals in Yuma (Arizona), Maricopa (Arizona), and Imperial Valley (California). All samples were assayed for total coliforms, Escherichia coli, turbidity, pH, temperature, conductivity, and salinity. Escherichia coli and total coliforms were enumerated in all water samples using Colilert Quanti-Tray® (IDEXX Laboratories, Westbrook, ME) most probable number (MPN) method following manufacturer instructions. All bacterial data were log transformed to minimize skewness. Pearson Correlation analysis was used to identify relationships between microbial concentrations and independent variables (e.g. physical, chemical, weather, canal discharge rates). A significance level cutoff of α=0.05 was used for all correlative statistical tests. Stata Statistical Software (StataCorp, College Station, TX) was used for traditional statistical analyses, including Two-sample t-tests and Analysis of Variance (ANOVA) and Kruskal-Wallis tests. Overall, there were 88 unique sites with an average of 15 samples per site. With respect to the latest Food Safety Modernization Act, two sites had Escherichia coli geometric means >126 MPN/100ml. When a statistical threshold value (STV) of 410 Escherichia coli MPN/100ml was applied to all sites, one site exhibited a 31% violation rate (12/38 samples). Across all sites, statistically significant correlations (p<0.001), although weak, were identified between Escherichia coli and air temperature (r=0.1414), water temperature (r=0.2472), relative humidity (r=-0.1799), pH (r=0.1521), and dissolved organic solids (r=-0.1888). Following the analysis of 1,367 samples for Escherichia coli and physical and environmental parameters, the following key irrigation water collection approaches are suggested: 1) Explore up to 600m upstream to ensure no major contamination or outfalls exists; 2) Sample before noon; 3) Collect samples at any point across the canal where safe access is available; 4) Collect samples at the surface of the water; and 5) Composite five samples and perform a single Escherichia coli assay. These recommendations consider the entirety of our data as well as sampling costs, personnel effort, and scientific knowledge of water quality characterization in the Southwest region. These guidelines will better characterize risks from microbial pathogen contamination in irrigation waters and aid in risk reduction practices for agricultural water.
**Water Quality Testing for Bacterial Indicators**

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Kellie Messer

Water Quality Testing for Bacterial Indicators Coliforms are bacterial indicators used to define the quality of water. While coliform bacteria are an important part of ensuring public health, coliform bacteria are not fully protective of public health (Plummer et al. 2014). There have been outbreaks of waterborne disease where coliform bacteria were not detected, and also cases where coliform bacteria were detected, but no pathogens were identified (2014). These cases occurred due to various issues, one being that viruses and bacteria are uniquely impacted by environmental conditions. Extremes in pH, salinity, and temperature will affect coliform bacteria to a higher degree than viruses (Fong and Lipp, 2005). Additionally, in groundwater wells it was found that the association between viruses and bacteria was not statistically legitimate (Borchardt et al 2003). Coliphages have the potential to yield higher success rates in detecting viruses in water in comparison to the inconsistencies of coliforms (Furuse, 1987). Our research will investigate the presences of coliforms and coliphages and will evaluate the correlations between their presence and various environmental conditions. This project includes environmental water samples from the Muddy River located in Back Bay Fens of Boston, Massachusetts. Water samples will be taken from multiple locations along the river during both the winter and spring seasons. Samples will be extracted from the river every two weeks and quantified for concentrations of coliforms (total, fecal and E. coli) and coliphages (somatic and male specific). Additionally, tests that will be conducted to describe environmental conditions including total solids, suspended solids, volatile solids, conductivity, hardness, BOD5 and presence of phosphorus, nitrate, and nitrite. It is expected that there will be high concentrations of the indicators because the Muddy River is located in a highly populated area and the river is home to many birds including a wide variety of ducks and geese all year round. Investigating the contents of the environmental water samples at the microbiologic level will enable a proper analysis of the interrelation between coliforms and coliphages.

**Handwashing for Ebola Outbreak Contexts: Comparison of Soap, Hand Sanitizer, and 0.05% NaDCC, HTH, and NaOCl Chlorine Solution on the Inactivation and Removal of Model Organisms from Hands**

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Background. To prevent disease transmission during an Ebola outbreak, frequent handwashing with soap and water, alcohol-based hand sanitizer, or chlorine is recommended in Ebola Treatment Units. The 2014 West Africa outbreak was the first widespread Ebola outbreak, and this recommendation was widely extended to community settings. However, organizations differed in terms of which of these methods of handwashing they recommended. These contradictory guidelines created confusion and demonstrate that little is known about best handwashing practices in this emergency context. We will evaluate the efficacy of six different handwashing methods (soap and water, alcohol-based hand sanitizer (ABHS), 0.05% NaDCC chlorine solution, 0.05% HTH chlorine solution, and both stabilized and
non-stabilized 0.05% NaOCl chlorine solutions) on the removal and inactivation of non-pathogenic model organisms from human hands. While work has been done on handwashing efficacy in the past, these studies neither address chlorine use nor estimate the efficacy of these methods for use in an Ebola context. Methods. Model organisms will provide an estimate of the sensitivity of the Ebola virus to all these handwashing methods while allowing for testing with human volunteers. Escherichia coli (E. coli, ATCC 25922) will be used as a reference method, and the Phi6 bacteriophage (HER #102) will be used as a viral indicator of pathogen inactivation/removal from hands. Phi6 was chosen as an appropriate model for the Ebola virus because it shared characteristics with the Ebola virus and demonstrated a resistance equivalent to or slightly greater than published results for Ebola in pretesting. We will recruit 18 volunteers to participate in testing, utilizing each organism both with and without an added tripartite organic load (composed of bovine serum albumin, tryptone, and bovine mucin) for a total of four test conditions per volunteer. The addition of organic load helps to simulate realistic conditions in which the virus is shed in bodily fluids that exert chlorine demand. For each handwashing method, hands will be inoculated with a mixture composed of a suspension of the test organism (68% of the inoculate) and buffer solution or soil load (32%) and then washed. After washing, hands will be rinsed using a modified glove juice method to retrieve any organisms remaining on the hands and results quantified by membrane filtration (E. coli) or small drop plaque assay (Phi6). The impact of each handwashing method is estimated by comparing the log reduction in organisms retrieved in each handwashing test to the log reduction in a control in which hands are inoculated but not washed. We will also collect rinse water to test for bacterial and viral persistence. We have both surrogate organisms in stock and have pretested methods with both organisms. We are awaiting a final decision from the IRB after responding to minor comments to begin testing. We anticipate testing will begin the final week of March and will last around 2-3 weeks. Results will be fully available at the time of the conference. Discussion and Conclusions. Quantifying the inactivation and removal of these model organisms after handwashing will allow us to estimate the sensitivity of the Ebola virus to each method. The data from this study will be considered along with data about the impact of the methods on the integrity of skin on hands and information about the context and challenges of providing different handwashing solutions to provide recommendations for hand hygiene practices in Ebola and similar emergency outbreak settings.

Effect of Environmental Factors on Coliphage Concentrations in Surface Waters

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Coliphages have been suggested as water quality indicators. The objective of this research was to evaluate effects of environmental factors on coliphage concentrations in San Diego surface waters using both field sampling of recreational waters and controlled mesocosm experiments. Water samples were collected from beach sites during rainfall and tidal events and analyzed for F+ and somatic coliphages, and from a controlled freshwater system in summer and winter. Regression models determined significance of coliphage concentration with different environmental factors. Coliphage concentrations were significantly affected by sample location, rainfall, water temperature, and season, but not by surf height, sea state, salinity, kelp coverage, tide height, wind speed, and turbidity (alpha = 0.05). Potential coliphage die-off was observed along the San Diego River. This research informs how
environmental factors affect coliphage concentrations and demonstrates timing and conditions for viral contamination of surface waters.