NARMS* One Health Metagenomic Surveillance of Antimicrobial Resistance in Water

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- Multi-agency collaboration for surveillance of AMR in water
- Optimization and synchronization of collection protocols and microbiological and molecular processing methods for interoperable data across global One Health surveillance efforts.

Abstract

Antibiotic resistance (AMR) has been described as the biggest public health challenge of our time – an issue made even more significant as we face a global pandemic, where recovery from Coronavirus Disease 2019 intersects with a complex array of secondary infections and immune and microbiome mediated factors. A prominent focus of the National Antimicrobial Resistance Monitoring System (NARMS) of the Center for Veterinary Medicine's One Health research program is the metagenomic, quasimetagenomic, genomic and microbiologic evaluation of water that intersects the human, animal and environmental health. Water is fundamental to healthy food, and antimicrobial resistance elements present in water are constituents of a baseline that is critical to surveil and steward. Collaborative work between FDA and USDA is optimizing sample collection protocols and laboratory and bioinformatic methods to identify resistance genes in environmental and food production water sources.

Mitigation of risks cannot be achieved without reliable data – generated by modernized and standardized collection protocols in collaborative interagency initiatives. Culture independent (metagenomic) profiles of water are being conducted in concert with quasimetagenomic (shotgun sequencing of microbiological enrichments) to identify the most practical approaches for provision of AMR data to underpin risk mitigation strategies. Here we provide side-by-side microbiome and resistome profiles for metagenomic and quasimetagenomic data to describe the resistome of water collected at the United States National Arboretum's Hickey Run.

DNA from water enriched in Universal Pre-enrichment Broth (UPB) and Modified Buffered Peptone Water (MBPW) (widely used first steps in the growth of *Salmonella*) and Modified Buffered Peptone Water with pyruvate (MBPWp) (first step in growth of *E. coli*), were contrasted with DNA from culture independent water samples. Not surprisingly, a more expansive array of resistance genes was observed in DNA from enriched water when contrasted with culture independent water. The differences between enriched resistomes and culture independent resistomes is important to consider as we optimize methods aimed at financially practical and ecologically comprehensive risk assessments of total AMR in water for synchronized One Health Surveillance.

FDA Mission Relevance

Antibiotic resistance has been described as the most significant public health challenge of our time. NARMS and the Center for Veterinary Medicine's One Health research program evaluates metagenomic, quasimetagenomic, genomic and microbiological approaches to the description of antimicrobial resistance in water (and other human, animal and environmental reservoirs) for improved One Health public health stewardship. We are working to identify the most practical, comprehensive and financially accessible approaches to describing the resistome of important human, animal and environmental reservoirs. We are working to streamline water evaluation across multiple agencies including (in the work presented here)- the United States Department of Agriculture and in ongoing planning efforts – the Environmental Protection Agency, the Centers for Disease Control and other offices at the Food and Drug Administration.

Methods

Samples were collected using standard 1 liter grabs to contrast with 10 liter and 50 liter ultrafiltration collections. DNA from backflush and filters were processed using Qiagen Power water kits according to the manufacturers specifications. Libraries were processed using Illumina DNAPrep and sequenced on a NextSeq 500 with the High Throughput kit.

Shotgun metagenomic and quasimetagenomic sequencing reads were quality trimmed using Illumina default parameters and fastq files were submitted to the Cosmos ID and AMR Plus Plus pipelines. AMR Plus Plus was used to process the raw shotgun metagenomic data, annotate sequences and count hits aligned to Antibiotic resistance (AMR) reference gene sequences (MegaRes v1.0.1). AMR ++ also provides a count of polymorphisms that occur for each aligned gene hit with respect to the reference database. As a result, count files were obtained for each sample that can be combined into a count matrix and analyzed using any statistical techniques that operate on a matrix of observations.

The community resistome was also described by querying the unassembled sequence reads against the CosmosID curated antibiotic resistance gene database. Antibiotic resistance genes were identified based on the percentage of gene coverage for each gene as a function of the gene-specific read frequency in each sample. The database has a number of kmers spanning the entire gene, these sets are interrogated and average frequency and percentage of total matches of all kmer hits are recorded.

Results

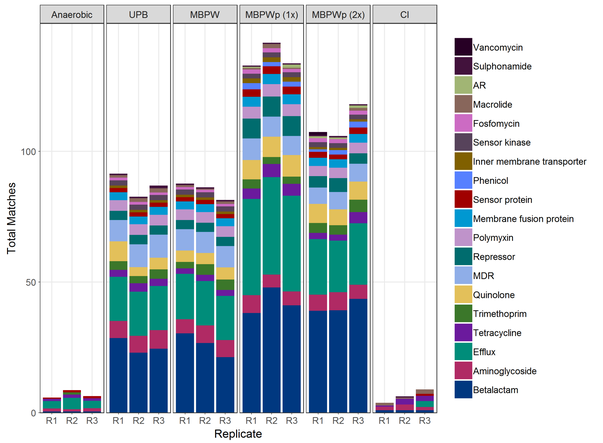

Figure 2. The relative abundance of AMR elements (resistome) in water that was enriched anaerobically (Anaerobic), for *Salmonella* with two different pre-enrichment broths: UPB (Universal Pre-enrichment Broth) and MBPW (Modified Buffered Peptone Water), and for *E. coli* with 1x and 2x MBPWp (Modified Buffered Peptone Water with pyruvate).

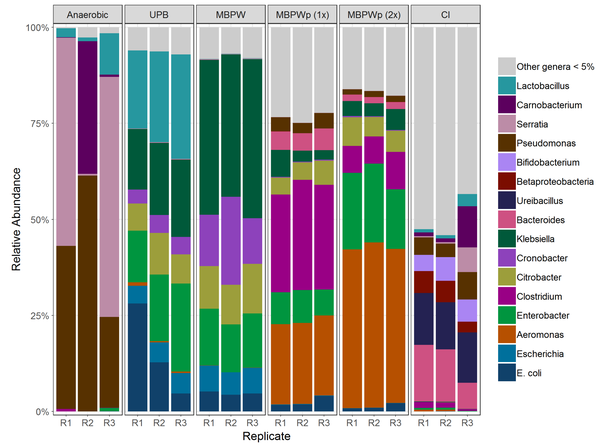

Figure 3. The relative abundance of prominent bacterial genera in replicates of each treatment. Each approach to culturing and culture independent examination of the water microbiome and resistome yields a diverse profile of the AMR potential and taxonomic diversity in a single water source.

Conclusions

Water Safety Modernization Approaches (WSMA) including new standardized collection, microbiological, molecular, and bioinformatic methods will be important to streamline as we plan the future interagency monitoring of the flow of AMR through human, animal and environmental reservoirs. The work presented here demonstrates that the quasimetagenomic approach (shotgun sequencing of pathogen enrichments) recovers 100 times more data to describe relevant living community resistance profiles. The development of standardized sampling methods for low, mid, and high volumes of water with concordant validation of recovery of pathogens and description of total microbiota and total antimicrobial resistance potential, will ensure highly accurate outbreak response, risk assessment and acquire the necessary data to identify emerging threats and modernize surveillance and preventative controls. Water has the potential to act as the source or route of contamination for introduction of pathogens to agricultural, environmental and urban systems. The comprehensive characterization of agricultural, urban and environmental water sources – from specific pathogen levels and culture independent (CI) microbial community composition to antimicrobial resistance profiles will be an integral part of understanding and predicting risks associated with water across a wide range of ecologies and applications. The assemblage of high resolution AMR data and standardized sampling approaches will support science - based modernization of surveillance and preventative control for a new era of One Health Monitoring and implementation of preventative controls.