Improved methodology for detection of antibiotic resistance mechanisms from wastewater for population surveillance

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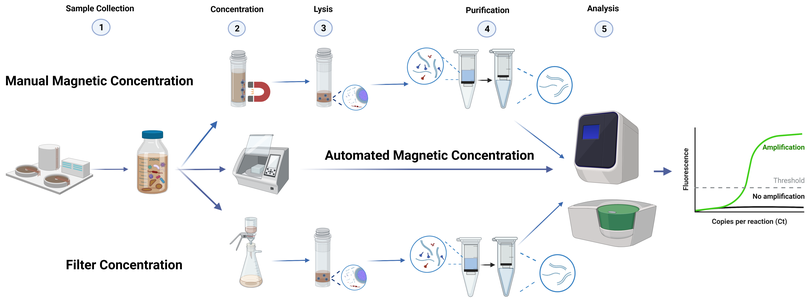
Background



COVID-19 highlighted the use of wastewater testing as a complementary method to monitor emerging outbreaks of infectious pathogens, including those harboring antimicrobial resistance (AMR) genes. Antibiotic resistant bacteria are identified as a primary public health concern that threatens to eliminate the effective use of antibiotics. As a strategy to complement population testing, wastewater samples can be evaluated to identify increases in the presence of AMR genes in communities. However, the use of molecular testing methods is challenging because many genetic mechanisms contribute to AMR, with the most common being β-Lactamases. To be an effective surveillance strategy, these tests must be designed to identify a broad range of targets associated with AMR, and the methods must be sensitive given the larger wastewater sampling volumes used for analysis. The data described here demonstrate methods for improved sample processing and a head-to-head comparison of commercially available real-time PCR-based assays to assess AMR burden in wastewater samples.

Scheme 1: Example workflows for processing wastewater samples and downstream nucleic acid isolation and analysis (created with BioRender.com).

Methods

Bacteria DNA Extraction: Bacteria from 10 mL wastewater samples were concentrated using Ceres Nanosciences Nanotrap® Microbiome Particles (B or A + B) or, for comparison, using the MagMAX™ filter concentration method. Nucleic acid extraction was done using the KingFisher™ Apex System with either the NucleoMag™ DNA/RNA Water extraction kit or the MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit.

Measurement of DNA Yield per Extraction: DNA concentration was quantified using a Qubit Fluorometer and the Qubit dsDNA HS Assay Kit, per manufacturer's instructions.

qPCR Analysis of Antimicrobial Resistance genes: Streck ARM-D® Kits and the BIOFIRE® FilmArray Torch system were used to detect AMR genes for sample addition and data analysis. ARM-D Kit analysis was done using a AB QuantStudio 7™ qPCR system.

Results

Combining Ceres Nanotrap Microbiome A + B Particles Improve DNA Yield from Wastewater Samples

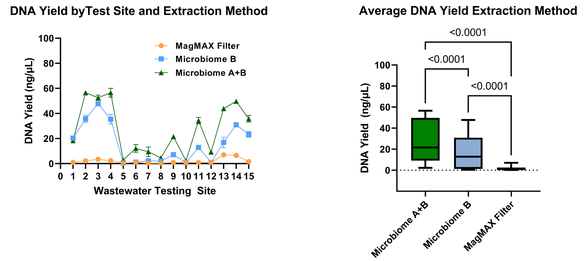


Figure 1. Compared to Ceres Microbiome B and ThermoFisher MagMAX Filter methods, combining Ceres Microbiome A and B Nanotrap particles resulted in the highest average total DNA yield per sample (n = 15 independent wastewater testing sites across the U.S.). The combination of Ceres Microbiome A and B particles is expected to collectively capture a larger number of pathogens in wastewater samples, accounting for the overall increase in total DNA yield.

Combining Ceres Nanotrap Microbiome A and B Particles Improve qPCR Detection of Bacteria

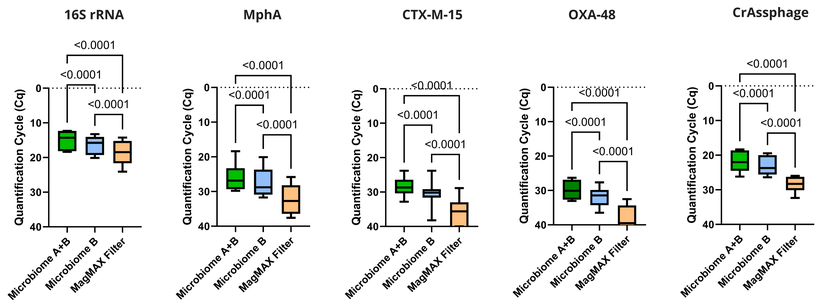


Figure 2. Influent wastewater, sampled from 15 independent U.S. wastewater testing sites, was concentrated using Ceres Nanotrap Microbiome particles and tested by custom qPCR assays (Ahmed et al., 2019). Combining Ceres Nanotrap Microbiome A and B particles resulted in increased qPCR assay sensitivity for 16S rRNA (bacteria), MphA (bacteria), CrAssphage (virus), CTX-M-15 (AMR), and OXA-48 (AMR) gene targets. Each respective target was detected 3 to 10 PCR cycles (i.e., 10 to 1000-fold) earlier than Microbiome B alone or the MagMAX Filter methods, which correlates well with the observed improvement to total DNA yield when Microbiome A and B particles are combined during the sample concentration step.

Results (continued)

Streck ARM-D Kits Identify Multiple AMR Genes in Concentrated Wastewater Extracts

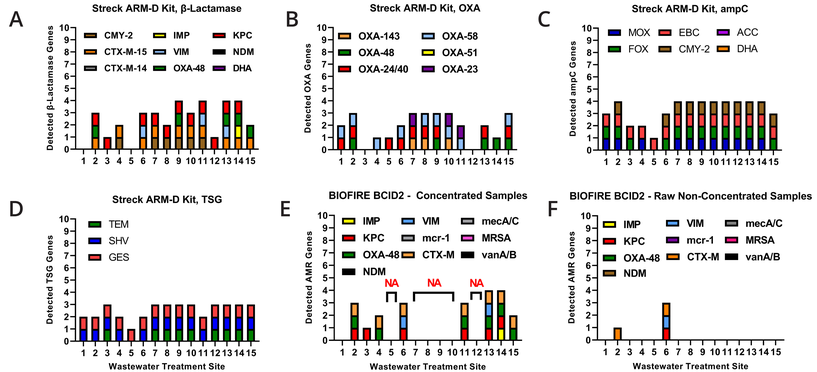


Figure 3. To screen for additional AMR mechanisms, the same 15 extracts from influent wastewater samples were evaluated by commercially available qPCR panels, the Streck ARM-D Kits and the BIOFIRE FilmArray BCID2 test. Results between the two qPCR panels were concordant for all but 3 samples; 2 OXA-48 variants were detected by the BIOFIRE BCID2 panel (samples 4 and 11, Graph A vs. Graph E) and a KPC was detected by the Streck kit (sample 12, Graph A vs. E), suggesting the samples do harbor these AMR mechanisms but differences in primer design may contribute to the variation in results for the three identified samples. For the BIOFIRE BCID2 panel, results depicted as not applicable (NA) represent sample runs where no organism, and thereby no AMR mechanism, was detected (Graph E). The Streck ARM-D Kits screen samples for a larger number of AMR genes (A, B, C, and D), resulting in identification of additional AMR mechanisms in these samples that were not identified with the BIOFIRE BCID2 panel. As expected, analysis of wastewater extracts not concentrated prior to analysis, resulted in a reduced number of detected AMR genes (F). Collectively, these data demonstrate use of Ceres Nanotrap Microbiome A and B particles, combined with the Streck ARM-D kits, provide an optimized workflow to screen for the broadest number of AMR variants in wastewater samples.

Conclusions

This data highlights the importance of combining an optimized sample concentration and extraction method with a robust molecular test capable of screening samples for many AMR mechanisms. Notably, evaluation of concentrated wastewater samples resulted in a 10- to 100-fold improvement in DNA yield and, consequently, increased qPCR assay sensitivity over the non-concentrated samples. Results between the Streck and BIOFIRE assays were concordant, with the exception that the Streck ARM-D Kits identified a broader number of gene targets. For example, 60% of samples were positive for OXA-58- and OXA-24/40-like variants and 40% of samples were positive for OXA-48-like variants. In total, 27 different AMR gene families were detected using these qPCR tests. Among the AMR mechanisms detected were IMP, KPC, and OXA-48, each classified as urgent or serious public health threats. Collectively, these results demonstrate the importance of selecting sensitive and specific methods to establish a consistent and reliable protocol for wastewater AMR surveillance. Overall, the combined methods permit effective detection of emerging antibiotic resistance threats aimed at improving infection control practices.