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

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Introduction

Antibiotic resistant bacteria, which harbor antimicrobial resistance (AMR) genes, pose a significant public health threat. For these bacteria, traditional means of antibiotic treatment are no longer effective. As such, it is imperative to monitor the prevalence of these bacteria. Recently, wastewater surveillance has been recognized as a complement to population testing that can 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 the ability of commercially available real-time PCR-based assays to determine AMR burden in wastewater samples.

Materials and Methods

Bacteria Concentration and DNA Extraction: Bacteria from 10 mL wastewater samples were concentrated using Ceres Nanosciences Nanotrap® Microbiome Particles (B or A + B) according to the manufacturer's instructions or by membrane filtration (Figure 1). Nucleic acids were extracted using the KingFisher™ Apex System (ThermoFisher Scientific) with the MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit (Thermo Fisher Scientific) (Figure 1).

Measurement of DNA Yield per Extraction Method: DNA concentration was measured using a Qubit™ Fluorometer (Thermo Fisher Scientific) with the Qubit™ dsDNA HS Assay Kit (ThermoFisher Scientific), per manufacturer's instructions.

qPCR Analysis of Antimicrobial Resistance genes: Streck ARM-D® Kits and the BIOFIRE® BCID2 Panel were used to detect AMR genes in DNA isolated from wastewater samples. The BCID2 Panel is not intended for use with wastewater samples but was utilized as a complementary screening tool for AMR genes. For the ARM-D Kits, an Applied Biosystems™ QuantStudio™ 7 qPCR system was used for analysis. The BCID2 Panel was run on the BIOFIRE FilmArray® System.

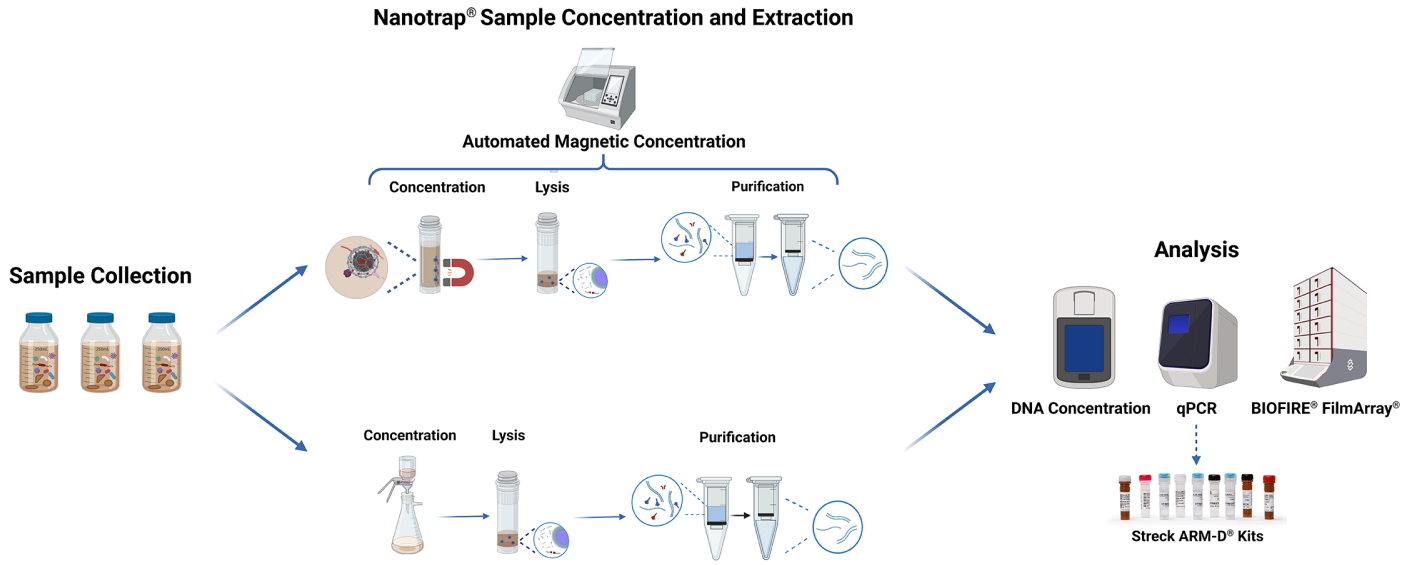

Figure 1. Workflows for wastewater sample processing. *Influent wastewater samples from 15 collection sites across the U.S. were concentrated using Nanotrap® Microbiome Particles or by membrane filtration prior to DNA extraction and purification. Isolated nucleic acid concentration was measured, and gene abundance was analyzed via qPCR or using the BIOFIRE FilmArray® System. For qPCR analysis of antibiotic resistance mechanisms, Streck ARM-D Kits were used. Created with BioRender.com.*

Results and Discussion

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

A major problem in wastewater surveillance is that large sample volumes typically dilute the small amounts of pathogens present in these samples. To address this issue, samples are often concentrated to enrich microbes and thus, to improve nucleic acid yield. However, traditional filter-based concentration methods often have multiple time-consuming steps and can produce inconsistent or low yields. Magnetic particle-based concentration provides a more rapid means of microbe enrichment. One such particle-based method uses the Nanotrap Microbiome Particles. Magnetic particle-based concentration, such as use of the Nanotrap Microbiome Particles, provides a more rapid means of microbe enrichment. To identify whether the Nanotrap Microbiome Particles are an efficient method of concentration, influent wastewater samples from 15 independent wastewater testing sites across the United States were concentrated by membrane filtration or with Nanotrap Microbiome Particles and DNA yield was measured. Samples concentrated by combining Nanotrap Microbiome A and B particles had higher average total DNA yield per sample than those concentrated with Nanotrap Microbiome B Particles alone or by membrane filtration (Figure 2). This overall increase in DNA yield suggests that Nanotrap Microbiome A and B particles collectively capture a larger number of microbes in wastewater samples.

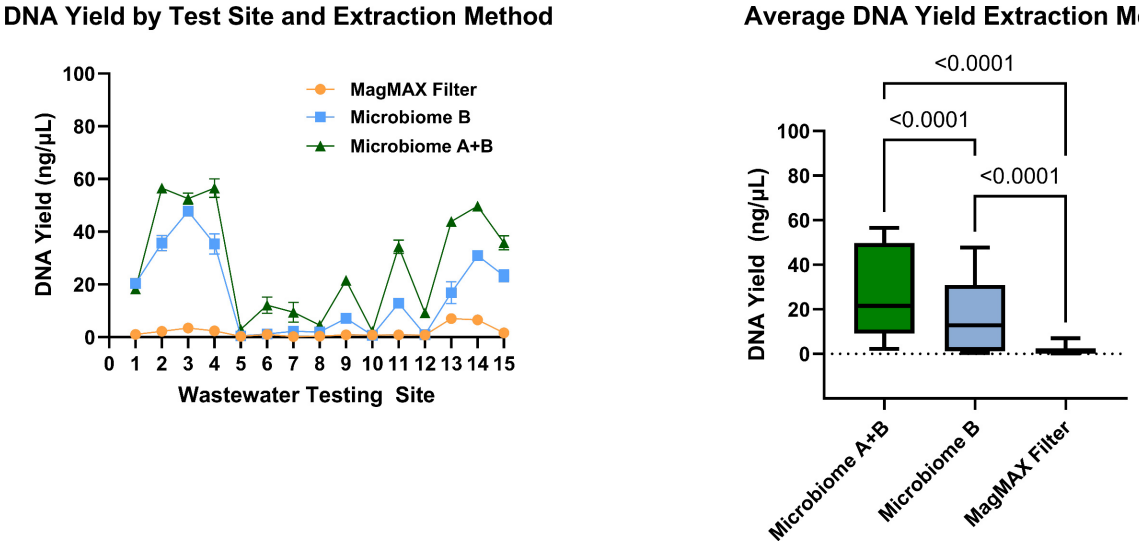

Figure 2. Concentration of wastewater samples with a combination of Nanotrap® Microbiome A and B Particles increases DNA yield. *Samples from 15 independent wastewater testing sites across the U.S. were concentrated and DNA was isolated using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit. Concentration methods include membrane filtration (MagMAX™ Filter), Nanotrap Microbiome B particles alone (Microbiome B) or a combination of Nanotrap Microbiome A and B Particles (Microbiome A+B). DNA yield per testing site and concentration method (left). Combined average DNA yield across all testing sites per concentration method (right). Means ± SD are shown (n=15 sites; 2 replicate concentrations and extractions per site). P <0.0001 by two-way analysis of variance (ANOVA).*

Combining Nanotrap Microbiome A and B Particles Improves qPCR Detection of Bacteria

To determine if the increase in DNA yield observed when the Nanotrap Microbiome A and B Particles are combined correlates with increased concentrations of pathogens in wastewater samples, we measured the amount of viral RNA and multiple bacterial DNA targets using custom singleplex qPCR assays (crAssphage, CTX-M-15, OXA-48, MphA, Ahmed et al., 2019; 16S rRNA, Streck ARM-D Kits). When samples were concentrated with Nanotrap Microbiome A and B Particles, 16S rRNA (fecal indicator for bacterial molecular targets), crAssphage (fecal indicator for viral molecular targets), CTX-M-15 (AMR), OXA-48 (AMR) and MphA (AMR), gene targets were detected 3 to 10 PCR cycles (i.e., 10- to 1000-fold) earlier than samples concentrated with Nanotrap Microbiome B Particles alone or by membrane filtration (Figure 3A-E). These data demonstrate that microbe concentration was improved when Nanotrap Microbiome A and B Particles were combined, which correlates with increased DNA yield and greater qPCR assay sensitivity.

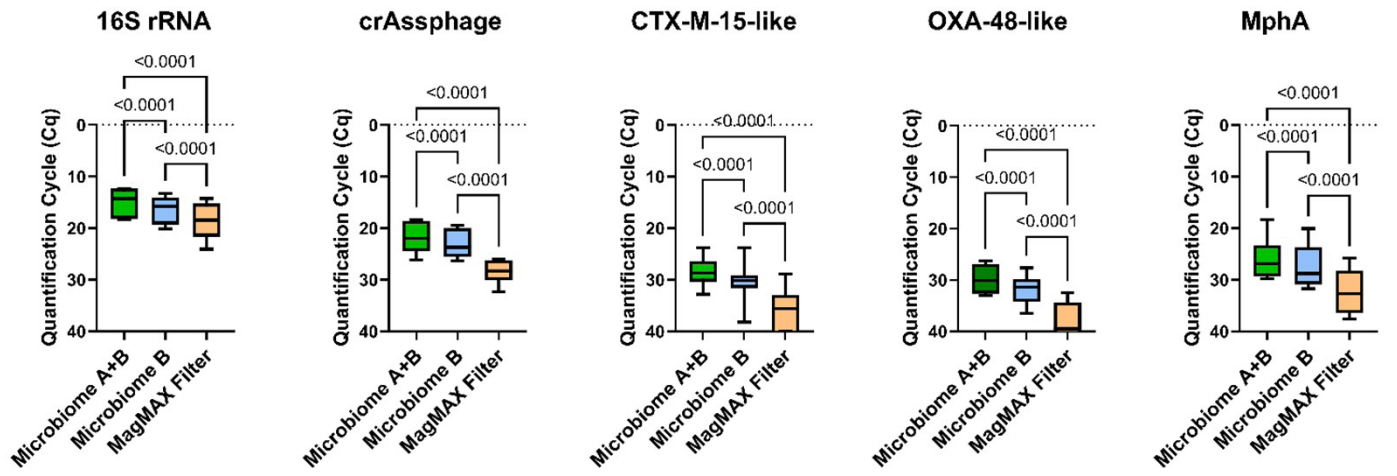

Figure 3. Samples concentrated with Nanotrap® Microbiome A and B Particles have improved qPCR detection of bacteria. *Samples from 15 independent wastewater testing sites across the U.S. were concentrated and nucleic acids were isolated using the MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit. Concentration methods include membrane filtration (MagMAX Filter), Nanotrap Microbiome B Particles alone (Microbiome B) or a combination of Nanotrap Microbiome A and B Particles (Microbiome A+B). Average Cq values for the 16S rRNA gene (A), crAssphage (B), CTX-M-15-like (C), OXA-48-like (D), and MphA (E) across all testing sites. Means ± SD are shown (n=15 sites; 3 PCR replicates per site). P <0.0001 by two-way analysis of variance (ANOVA).*

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

While our data supports the ability of Nanotrap Microbiome Particle-based capture and concentration to increase qPCR detection of AMR genes, robust surveillance would monitor for several additional AMR mechanisms. To determine if a wider breadth of AMR genes could be identified from concentrated wastewater samples, we examined commercially available qPCR panels from two vendors, the Streck ARM-D Kits and the BIOFIRE FilmArray® BCID2 test. We observed concordant results between the two qPCR panels for all but 3 samples; 2 OXA-48 variants were detected by only the BIOFIRE® BCID2 panel (Figure 4A vs. 4E, samples 4 and 11) and a KPC variant was detected by only the Streck kit (Figure 4A vs. 4E, sample 12). The variation in results for the three identified samples may be due to differences in primer design, as the data suggest that the samples do harbor these AMR mechanisms. For the BIOFIRE BCID2 panel, results depicted as not applicable (NA) represent sample runs where no organism, and thereby no AMR mechanism, was detected (Figure 4E). Because the Streck ARM-D Kits screen samples for a larger number of AMR genes (Figure 4A-D), additional AMR mechanisms were identified in the 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 (Figure 4F). Collectively, these data demonstrate that use of Nanotrap Microbiome A and B Particles in conjunction with Streck ARM-D kits provides an optimized workflow to screen for the broadest number of AMR variants in wastewater samples.

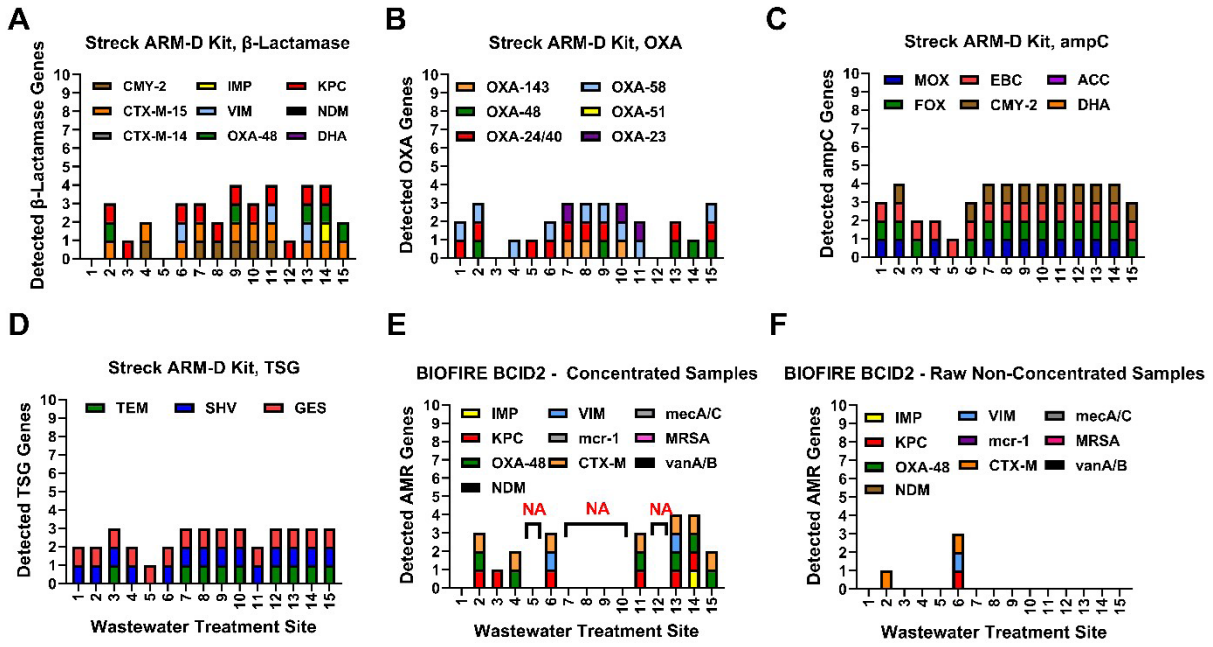

Figure 4. Comparison of AMR gene identification by Streck ARM-D® Kits and the BIOFIRE® BCID2 Assay. *Samples from 15 independent wastewater testing sites across the U.S. were concentrated using Nanotrap® Microbiome Particles (A-E) or were not concentrated (F), and AMR genes were identified using Streck ARM-D Kits—β-lactamase (A), OXA (B), ampC (C), TSG (D)— or the BIOFIRE BCID2 Assay (E and F). NA, no organism detected.*

Conclusions

Our 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. Concentration of wastewater samples with Nanotrap Microbiome Particles resulted in a 10- to 100-fold improvement in DNA yield and consequently increased qPCR assay sensitivity over non-concentrated samples. When concentrated samples were assayed for AMR mechanisms, the Streck ARM-D Kits and the BIOFIRE BCID2 assay collectively detected 27 different AMR gene families, including IMP, KPC and OXA-48, which are classified by the CDC as urgent or serious public health threats. Notably, the Streck ARM-D Kits identified a broader number of gene targets than the BIOFIRE BCID2 assay. Of the samples assayed, 60% were positive for OXA-58 and OXA-24/40-like variants, and 40% were positive for OXA-48-like variants. Taken together, these results demonstrate the importance of selecting sensitive and specific methods to establish a consistent and reliable protocol for wastewater AMR surveillance. The combination of Nanotrap Particle-based capture and concentration with qPCR analysis using the Streck ARM-D Kits is an effective means of detecting emerging antibiotic resistance threats, allowing for the implementation of improved infection control practices.