Antibiotic Resistance Genes in Marquette County Wastewater

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Abstract

Infections by antibiotic-resistant bacteria increase the severity and virulence of the disease and decrease the host's ability to fight the infection, leading to increased patient morbidity and mortality. Currently, while multiple wastewater Antibiotic Resistance Gene (ARG) studies have focused on rural communities, comparatively few ARG wastewater studies have focused on rural communities in the United States. This study focuses on the presence of ARGs in the wastewater from a rural community in the Upper Peninsula of Michigan.
Following treatment with a specific antibiotic, resistant bacteria persist and reproduce, creating offspring that are also resistant to the antibiotic. The objective of this study was to determine which ARGs are currently in the Marquette community. Samples from the wastewater treatment plant and a sewage waste station close to the hospital were collected to assess the presence of ARGs in Marquette county as a whole, and the hospital neighborhood, respectively.
A Qiagen Microbial DNA qPCR plate was used to screen for 87 different ARGs to detect the presence or absence of a given gene. After determining which ARGs are present, digital droplet PCR (ddPCR) was used to quantify the specific levels of ARGs identified.
The initial Qiagen screen revealed the presence of 41 different ARGs, most of which encoding resistance for beta-lactams. This data was validated for 10 different ARGs using ddPCR, including bla_{CTX-M}, bla_{TEM}, bla_{CMY-2}, bla_{SHV}, KPC, NDM, OXA-48, VIM, vanA, and mcr-1. Of most note was the detection of carbapenemase encoding genes. Carbapenemases are enzymes that inactivate carbapenems, which are β-lactam antibiotics used to treat multi drug-resistant infections. These enzymes allow the bacteria to become resistant to a plethora of antibiotics. These samples were collected quarterly, and screened for the presence of the above ARGs.

Because carbapenemase genes were detected in the wastewater, it became prudent to identify which bacteria are harboring these genes. Bacteria from the wastewater were isolated using agar plates made with antibiotics (ertapenem and meropenem). Ertapenem and meropenem resistant bacteria were isolated, and screened for KPC using ddPCR. Bacteria isolates were also screened for the expression of the 5 major carbapenemases; KPC, OXA-48, VIM, IMP, and NDM using the CARBA-5 NG test strips from Hardy Diagnostics.
Chromosomal DNA from these isolated bacteria was extracted using a Qiagen DNEasy Ultraclean microbial kit, and any potential plasmids were isolated using an Invitrogen PureLink plasmid kit. The chromosomal DNA samples were then sent out for 16s sequencing to determine the identity of the bacteria harboring carbapenem resistance. This information could be valuable for public health authorities to assess the risk of spread of antibiotic resistance in communities. It may also influence the antibiotics chosen by community physicians to treat infections.

Why wastewater?

Figure 1: Wastewater Treatment Plants are common places to look for ARGs because a multitude of antibiotics are disposed of into the wastewater. The above figure demonstrates how the wastewater treatment plant gains antibiotic resistance genes from many different sources, including agriculture and healthcare settings.

Methodology

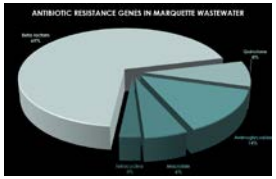


Figure 2: Our first step was to screen for the presence / absence of a variety of ARGs using Quantitative PCR (qPCR) and the Microbial DNA plate from Qiagen. This 96 well plate contained primer/probe pairs for 88 different ARGs and controls. Of the 88 ARGs screened our results found, 41 ARGs screened positive, and 47 screened negative. The highest quantity of ARGs present were in the Beta-lactams category. A breakdown of the ARGs detected in Marquette wastewater are shown above. Of most concern was the detection of ARG encoding Beta-lactams and specifically those encoding carbapenemases, the genes bla CTX-M , bla TEM , bla CMY-2 , bla SHV , and KPC were detected. The next step was to quantify these results using, digital droplet PCR (ddPCR).

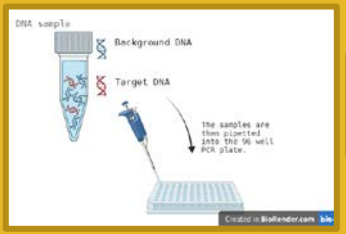
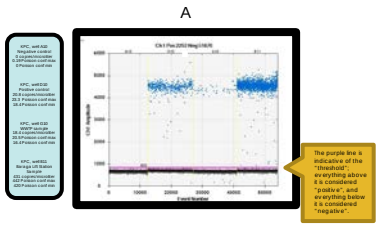
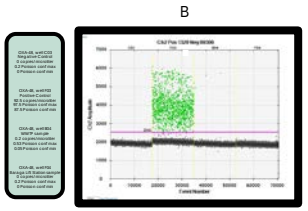


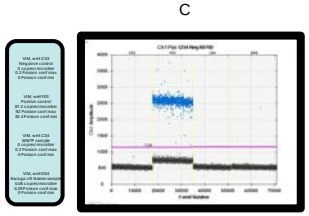
Figure 3: The initial process of Droplet Digital PCR (ddPCR) is similar to a typical PCR reaction. Primers and probes are added to a PCR master mix and template DNA is added. The template DNA will be screened to quantify the target DNA in the sample.
To start, the sample is pipetted in triplicate into a 96-well plate.

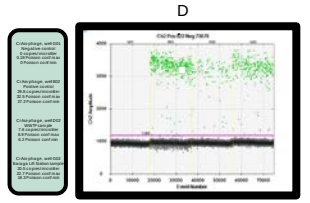
Figure 4: The plate is then put into the droplet generator, in which the sample is then partitioned into 10,000+ uniform nanodroplets of oil. The oil droplets containing PCR reaction mix are placed into a thermal cycler to amplify target sequences.
Following thermocycling, the droplets are run through a droplet reader that measures the fluorescence of each individual droplet. The reader separates the droplets into positive or negative droplets based on the amount of fluorescence. The machine reads the results as the ratio of positive droplets to total droplets, and it can determine the quantity of target DNA in the starting sample. ddPCR is more advantageous in this study than qPCR because ddPCR has increased sensitivity.

Results









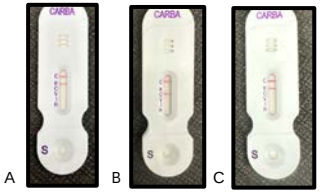


Figure 6: Following ddPCR, we grew bacteria on TSA or HK agarplates made with 2mcg / mL of ertapenem. For those bacteria that grew, we isolated and tested for the expression of 5 main carbapenemase proteins using the Carba-5-NG strips pictured above. 5A depicts the positive control. 5B depicts a positive OXA-48 protein detection. 5C depicts a positive KPC protein detection.

Figure 5: This figure shows the data obtained from ddPCR. Quantities of positive droplets are denoted in the boxes on the left.

Moving forward

Carbapenemase-producing bacteria were sent out for 16S sequencing. Initial findings indicated the presence of Pseudomonadaceae, Aeromonas, and Enterococcus. However, none were specific enough to identify the species level. Long read 16S sequencing is currently being completed to hopefully obtain more specific identification of the carbapenemase-expressing bacteria. A better understanding of which ARGs are present in the community and which bacteria are harboring these genes has the potential to inform public health authorities and clinicians about the threat of antibiotic-resistant infections and the spread of ARGs. Ultimately this type of research could lead to better antibiotic stewardship and patient care in a community.