An Evaluation of Antibiotic Resistance in Bacteria of the White River Waterways: Water Sampling (Poster 1)

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Abstract

The dramatic increase in the prevalence of antibiotic resistant (AR) genes in numerous bacterial species has been a heavily discussed topic within the scientific community. Antibiotic resistant bacteria pose a serious threat to an individual's health and is an ongoing area of research with a variety of public health implications. Antibiotic resistant genes can lead to the development of "super bugs" which can become immune to currently used antibiotics. This leads to much more severe infections, difficulties in properly treating those infections, and can result in long-lasting health complications.

There are several factors responsible for the development of these "super bugs," namely over prescription and the spread of medical and agricultural runoff in natural environments. This runoff exposes bacteria to antibiotics and allows them to develop resistance and spread rampantly between non-pathogenic and pathogenic bacteria.

Identifying local reservoirs that house these AR bacterial strains has become pertinent. The white river watershed flows through thousands of miles of streams and local drainage areas providing drinking water to residents of central Indiana. The Nina Mason Pulliam Ecolab (NMPE) at Marian University is part of this specific watershed. This Ecolab provides a prime environment in which different bacterial strains can thrive. This provides a crucial medium for the mixing, evolution, and spread of antibiotic resistant genes and bacteria. Gathering and studying water samples from the NMPE will provide insight into the prevalence of multidrug resistance genes. Previous research at MU-COM has found bacteria carrying multiple drug resistance (MDR) in the Nina Mason Pulliam EcoLab (NMPE) watershed. In the summer of 2020, 87.5% of the samples collected exhibited multiple drug resistance.

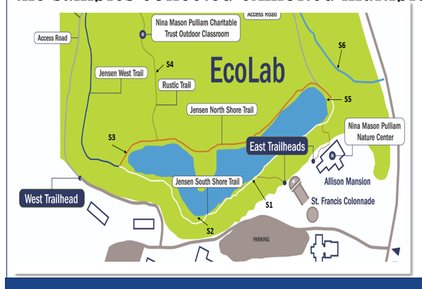

Fig. 1. Map showing Nina Mason Pulliam EcoLab, the source of water collected.

Objective

The main objective of this project was to gather pure bacterial isolates from the NMPE to further identify pathogenic gram-negative bacteria, test their resistance to a variety of antibiotics, and sequence the antibiotic resistant genes they contain. The results of this study will help create a resistance profile to serve as a guide for creating and implementing water management protocols.

Methods

Water Collection:

Approximately 500 mL of water was collected using an autoclaved one-liter glass bottle. Water samples were obtained every two weeks from an overflow drain to minimize sediment.

Filtration:

200 mL of water was filtered at a time using a vacuum filtration pump, shown below in **Fig. 3**. Filter papers of decreasing pore size (beginning with 10 µL and ending at 0.6 µL) were used to collect bacteria present. These filters were transferred to Mueller Hinton (MH) agar plates and incubated at either 37°C or 30°C for both 24 and 48 hours to isolate slow and fast-growing bacteria.

Isolation:

After incubation, bacteria grown on the plates were streaked onto MacConkey Plates for isolation of gram-negative bacteria. Subculturing was repeated until isolated colonies were attained.

Gram-staining:

The subculture plates were routinely examined using gram-staining to identify colony morphology and the progress in attaining pure isolates.

Glycerol Stocks:

Each isolate was inoculated in 2 mL of MH broth and placed in a shaking 37°C incubator for 24 hours. 850 µL of each culture was added to 150 µL of sterilized glycerol in glycerol stock tubes. These tubes were frozen in the -80°C freezer. Each sample was then streaked onto MH agar plates to be sent out for sequencing and identification.

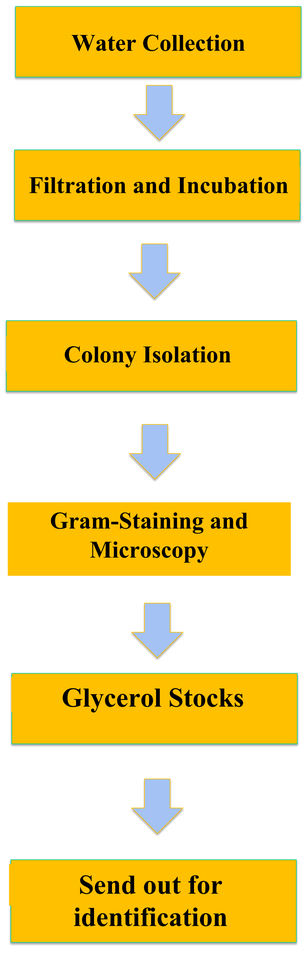

Fig. 2. Flow diagram giving an overview of the sampling and data collection procedure.

Fig. 3. The vacuum filtration pump used to collect bacteria from water samples

Results

Isolate #	Gram +/-	ID and shape
1.1	negative	*Enterobacter* spp; *Pantoea* spp. Rod-shaped
1.2	negative	*Enterobacter* spp; *Pantoea* spp. Rod-shaped
1.3	negative	*Enterobacter* spp; *Pantoea* spp. Rod-shaped
2.1	negative	*Enterobacter* spp; *Pantoea* spp. Rod-shaped
4.1	negative	*Enterobacter* spp; *Pantoea* spp. Rod-shaped
4.2	negative	*Enterobacter* spp; *Klebsiella aerogenes*. Rod-shaped
6.1	negative	*Enterobacter* spp; *Klebsiella aerogenes*. Rod-shaped
6.2	negative	*Enterobacter* spp; *Pantoea* spp. Rod-shaped
13.2	negative	*Enterobacter* spp; *Leclercia adecarboxylata*. Rod-shape
13.3	negative	*Enterobacter* spp; *Leclercia adecarboxylata*. Rod-shape
15.3	negative	*Enterobacter* spp; *Leclercia adecarboxylata*. Rod-shape
15.4	negative	*Enterobacter* spp; *Leclercia adecarboxylata*. Rod-shape
16.2	negative	*Enterobacter* spp; *Leclercia adecarboxylata*. Rod-shape

Fig. 5. A small sample of isolates gathered and their identification

- Fifty-five bacterial isolates were sent for sequencing. A sample of the resulting data is shown above in **Fig. 5**.
- BLAST analysis of the isolates showed that the entirety of the isolates were gram-negative and members of the *Enterobacteriaceae* family of bacteria.
- *Enterobacter* species got the highest scores in BLAST analysis but it also picked up other members of the *Enterobacteriaceae* family including *Serratia*, *Citrobacter* and *Klebsiella*
- One possibility is that the location from which water was sampled had a high concentration of the *Enterobacter* species
- A more precise ribosomal RNA homology search database will confirm the identity of the isolates

Discussion

- All the bacteria isolated belong to a family that has many human pathogens. For example:
  - *Klebsiella aerogenes*, a subspecies of *Klebsiella pneumoniae* is a prominent pathogen of multi-drug resistance pneumonia that is responsible for hospital outbreaks worldwide
  - *Pantonea* species have been identified as causative agents of infection in children and elderly and responsible for outbreaks of sepsis in NICUs
  - *Leclercia* species have been reported as an opportunistic pathogen in immunocompromised hosts
- The isolated bacterial samples obtained from the Ecolab are going to be evaluated for the degree of antibiotic resistance using Kirby Bauer assay
- Resistance genes will be identified with PCR and sequence analysis and it will be determined whether they are expressed on a plasmid or the genome

Acknowledgements

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