Confirmation of the Presence of Antibiotic Resistance Gram-Negative Bacteria in the Nina Mason Pulliam EcoLab (NMPE) by Kirby Bauer Assays and Genome Sequencing

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INTRODUCTION/ OBJECTIVE

Antibiotic resistant (AR) bacteria pose a serious threat to an individual's health, and the presence of AR genes can lead to the development of "superbugs" that are resistant to currently used antibiotics. Identifying local reservoirs that house AR bacterial strains has become critical. The main objective of our project was to identify the presence and cause of bacterial resistance in gram-negative bacteria collected from Jensen Lake at the Nina Mason Pulliam Ecolab (NMPE). This research was conducted via two protocols. To test for AR, gram-negative bacteria isolated from the NMPE water samples were subjected to Kirby-Bauer assays (Fig. 1). To test for the presence of AR genes, genomic DNA was purified from the bacterial isolates and sent to an outside vendor (ID Genomics) for identification of potential AR genes through PCR amplification and sequencing (Fig. 2).

METHODS

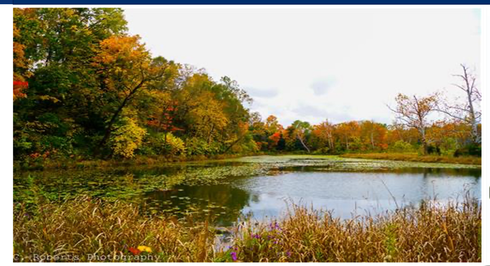
Kirby Bauer

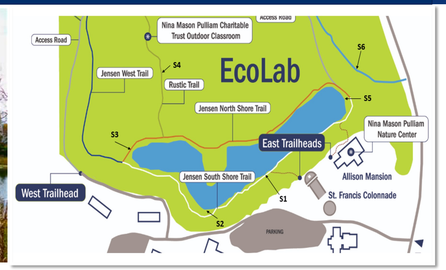
- Bacterial isolates grown overnight were inoculated into Mueller Hinton (MH) broth and grown at 37˚C, then shook at 250 rpm for a few hours
- Isolates were then diluted to the OD of 0.107 and swabbed onto MH agar
- Nine antibiotic disks were dispensed on the plates, and the plates were incubated for 24 hours at 37˚C
- Zones of inhibition were measured the next morning to the nearest millimeter and compared to CLSI standards to determine susceptibility or resistance to tested antibiotics

DNA Extraction

- Bacterial isolates were grown overnight in 1.5mL Luria-Bertani broth at 37˚C and shook at 250 rpm
- DNA extraction was performed using the GenElute Extraction Kit from Sigma
- Concentration of purified DNA was measured using nano drop and gel electrophoresis
- DNA samples were shipped to ID Genomics facility for identification of AR genes

ENVIRONMENT





Nina Mason Pulliam EcoLab

RESULTS

Amino-Glycosides	Beta-Lactams	Cephalosporins (1st, 2nd, & 3rd gen)	Carbapenems	Quinolones	Sulfonamides	Tetracyclines and Choramphenicol
Class: 62 (18.9%)	Class: 126 (77%)	1st: 47 (57%) 2nd: 31 (38%) 3rd: 30 (18%)	Class: 40 (49%)	Class: 62 (38%)	Class: 27 (17%)	Class: T: 8 (10%) C: 6 (7%)

Table 1. Number and Percentage of bacterial isolates with resistance to different antibiotic classes

- Of the 82 isolates tested, all showed resistance to at least one antibiotic. 80 of the 82 isolates showed multi-drug resistance.
- Isolates were most resistant to beta-lactam antibiotics (i.e. ampicillin, amox/clav) with 77% of strains having resistance to one of the two.
- Other notable individual antibiotic resistances were: cephalothin (73%), ciprofloxacin (43%), imipenem (43%), and streptomycin (37%).
- PCR and sequencing analyses showed antibiotic resistance genes in the 130 isolates tested: *CMY* (53), *CTX-M, TEM* & *SHV-1* (13 each) were the most common genes seen.

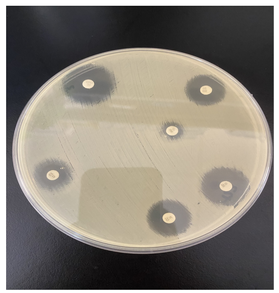


Fig. 1. Example of a Kirby-Bauer assay

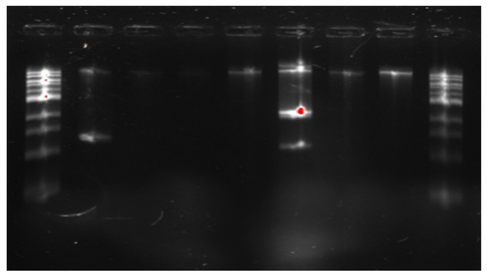


Fig. 2. An example of PCR amplification of the 16S rRNA gene of the bacterial isolates from Jensen Lake at NMPE. The 894 bp 16S rRNA gene product was amplified using universal primers. The 1kb DNA ladder was run in lanes 1 and 9 of the gel.

SUMMARY

- Our current data shows that many of the bacterial isolates from the NMPE display resistance to commonly used antibiotics in the medical field, most namely Beta Lactams.
- Based on the PCR and sequencing analyses of the strains isolated thus far, 108 samples have been identified to have a gene associated with antibiotic resistance.
- More genetic testing is underway to identify additional antibiotic resistance genes based on the Kirby-Bauer assay data.
- Further research is needed to locate whether these antibiotic resistance genes are encoded on the genomel, plasmids, or transposons.
- The long-term focus of our research is to examine the dissemination of AR genes in Indiana waterways and to document the presence of AR throughout different regions of Indiana.
- We also plan to test the levels of different classes of antibiotics in these water samples to see potential environmental exposures leading to increased rates of resistance.
- The focus on future research will also be to determine what the routes of dissemination are that lead to antibiotics being found in the waterways of Indiana.

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