

# The Increase of Antibiotic Resistance and its Implications in the Nina Mason Pulliam EcoLab

Stephanie Brown, Madison McReynolds, Azeem Ahmad\*, and Samina Akbar\*

College of Osteopathic Medicine and College of Arts and Sciences, Marian University, 3200 Cold Spring Road, Indianapolis, IN 46222 USA

\*Co-Investigators

Contact:  
Azeem Ahmad, College of Arts and Sciences, Marian University, 311-655-6274, aahmad@marian.edu  
Samina Akbar, Marian University College of Osteopathic Medicine, 311-655-6668  
sahmad@marian.edu  
Stephanie Brown, sbrown001@marian.edu  
Madison McReynolds, madisonm@marian.edu

## QUESTIONS

Question 1: Are bacteria resistant to clinically relevant antibiotics prevalent in the Nina Mason Pulliam EcoLab (NMPE)?

Question 2: How common is multi-drug resistance (MDR) to these antibiotics?

Question 3: Are there high numbers of gram-negative bacteria expressing resistance to more than one antibiotic?

## INTRODUCTION

Antibiotic resistance is a major and spreading issue capable of rendering antibiotics commonly used in clinical settings ineffective (Pourmand et al., 2017). Over prescription and the spread of medical and agricultural runoff has led to antibiotic accumulation in natural environments, exposing native bacterial populations to antibiotics and therefore promoting the development and spread of antibiotic resistance (Demirjian et al., 2015, Pruden et al., 2013). As a result, a reservoir of uncharacterized resistances can build in contaminated environments where non-pathogenic bacteria reside, allowing resistance to spread between non-pathogenic and pathogenic bacteria in an uncontrolled manner.



Fig. 1. Map showing the EcoLab and Lake Jensen, the source of water collected.

The Nina Mason Pulliam EcoLab (NMPE) contains 55 acres of land and is committed to environmental preservation. In past years, our lab has found bacteria carrying antibiotic resistance (AR) and MDR have been found in large quantities in the NMPE watershed, which feeds into the White River and Indianapolis watershed. We are looking to determine what impact these bacteria have had on the ecosystem and the source of their resistances. We seek to identify potentially pathogenic gram-negative bacteria and test their resistance to a variety of antibiotics.

Despite the growing threat of antibiotic resistance, little work has been done to characterize how these resistances spread between bacteria in natural environments, especially in urban ecosystems. In the greater Indianapolis watershed, antibiotic resistances have already been detected in a wide range of bacterial phyla, indicating that resistance can be held and transferred by non-pathogenic bacteria. In our previous work, we showed that 65% of environmental isolates show at least one antibiotic resistance, and 19 total bacterial genera carried resistance. Current findings indicate a lasting presence of diverse bacteria exhibiting AR and MDR. By cataloging all known resistances and their mode of transfer, a resistance profile can be constructed to guide medical treatment and water management protocols.

## METHODS

### Water Collection:

Approximately 500mL of water was collected using a one-liter glass bottle. Water samples were obtained from an overflow drain where less water sediment was present.

### Filtration:

The water was then filtered 50mL at a time using a 0.22 micron filter to collect bacteria present. These filters were transferred to MacConkey agar plates and incubated at 37°C for 24 hours.

### Isolation:

After incubation, bacteria grown on the plates were streaked out on new MacConkey plates for isolation. Iso<sup>+</sup> colonies were Gram stained. Using a premade kit, DNA was isolated. Frozen stocks of samples were made by combining 250 microliters of sterile glycerol with 750 microliters of bacteria/Mueller Hinton broth (made by combining 1.5mL of broth with a bacterial colony and motion-incubating overnight). Samples were characterized further via Gram staining and microscopy.

### Antibiotic Resistance Testing:

Liquid antibiotics were spread onto Mueller-Hinton agar and dried for ten minutes. One colony of bacteria was spread over the antibiotic-covered plate and allowed to incubate at 37°C for 24 hours before checking for growth. Antibiotic resistance or susceptibility was determined visually by observing areas of growth or no growth on the agar where antibiotic was spread. Antibiotics tested that were determined to be clinically relevant include gentamicin, sulfamethoxazole, cefoxitin, cephalothin, and ceftriaxone.



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

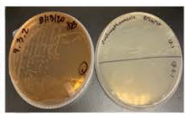


Fig. 4. Single colony isolation on MacConkey agar (left) and antibiotic resistance testing on Mueller Hinton agar (right)

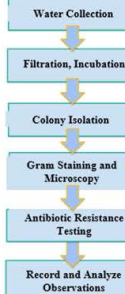


Fig. 2. Flow diagram giving an overview of the sampling and data collection procedure.

## RESULTS

Isolate #	Gram St.	ID	Cephalothin Resistance	Ceftriaxone Resistance	Cefoxitin Resistance	Sulfamethoxazole Resistance	Gentamicin Resistance
1.1	negative	000000001C1R	+	+	+	+	+
1.3.3	negative	000000001C1R	+	+	+	+	+
1.3.4	negative	000000001C1R	+	+	+	+	+
2.2	negative	000000001C1R	+	+	+	+	+
5.1.2	positive	ba011	-	-	-	-	-
5.2.3	positive	000000001C1R	-	-	-	-	-
8.2	negative	000000001C1R	-	-	-	-	-
9.1	negative	000000001C1R	-	-	-	-	-
10.3	negative	000000001C1R	-	-	-	-	-
10.1	negative	ba011	-	-	-	-	-

Fig. 5. A small sample of isolates gathered and their respective antibiotic resistances. While only focused on gram-negative bacteria currently, a gram-positive isolate was included to show the wide variety of bacteria exhibiting resistance.

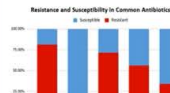


Fig. 6. Percentage of samples showing resistance and susceptibility to the five antibiotics tested. Data is representative of 32 samples collected and tested in June through September of 2020.

## DISCUSSION

Antibiotic resistance has shown to be a continuing issue within bacterial populations of the NMPE. All but two new isolates showed resistance to at least one of the antibiotics tested, and a vast majority of isolates showing resistance exhibited MDR. Resistance was most common in Cephalothin, a first-generation cephalosporin deemed broad-spectrum and used to treat gram-positive and some gram-negative mediated bacterial infections. This is believed to indicate that antibiotic resistance is common and widespread among bacteria in the population of the EcoLab, and likely throughout other populations in urban environments. We are in the process of doing 16S gene sequencing of all strains to identify genera. Further studies are needed to determine whether these resistance genes reside on mobile genetic elements or whether resistance is chromosomal and can be mobilized. While this pilot study is aimed to gauge the level of antibiotic resistance in the EcoLab specifically, it can be expanded upon by looking at other metropolitan environments in which runoff is prevalent. Ultimately, this data can influence the creation of new guidelines specifying how waste is contained and where it is allowed to flow.

## KEY POINTS

- Uncontrolled AR and MDR is a threat to animal and human health.
- MDR was present in 87.5% of samples collected in this summer of 2020.
- Bacteria showed some degree of resistance to all antibiotics tested.
- Building a resistance profile is critical to the development of safety guidelines for urban waste and runoff management.