Ohio River Water: Creating a Selective Environment for Pathogenic Bacteria

Mary Shea Ballantine

Determine if Ohio River water media:

- impacts the growth of *S. aureus* and *E. Coli.*
- encourages bacteria to turn on pathogenic genes: 1) antibiotic resistance and 2) larger biofilm formation.
- allows bacteria to gain antibiotic resistance in real-world conditions.

- **Growth of non-pathogenic bacteria decreased** when exposed to Ohio River water media: *S. Aureus* – by 6.08%; *E. coli* – by 17.3%.
- **Non-pathogenic bacteria created pathogenic traits** in presence of Ohio River water media: 1) Antibiotic resistance – zones of inhibition decreased compared to control: *S. aureus*—by 33.3% (Cipro and Chloramphenicol); and *E. coli*—by 39.14% (Cipro); by 36% (Chloramphenicol). 2) Biofilm formation increased: *S. aureus* – 3.72% larger BFI; *E. coli* -187% larger BFI.
- **Real-world conditions created antibiotic resistance** (exposed bacteria to Ohio River water for three days): *S. aureus*—21.8% decrease (Cipro); 25.4% decrease (Chloramphenicol); *E. coli*—26.06% decrease (Cipro); 38.7% decrease (Chloramphenicol).

- **12-hour growth curve** – bacteria exposed to Ohio River water media and to control media. Growth measured with Spec 20 every 20 minutes for 12 hours.
- **Antibiotic resistance tests** – 1) bacteria exposed to Ohio River water media and to control media, then spread on agar plates. Antibiotic disks (Ciprofloxacin and Chloramphenicol) added to each plate; incubated 12 hours; zones of inhibition measured.
- 2) Biofilm test – Expose bacteria to Ohio River water media or control media, incubate for 24 hours; measure biofilm formation using a Spec 20.
- **Real-world conditions** - Repeat antibiotic disk process using river water, not media.

Ohio River water created a selective environment for bacteria with pathogenic traits. It is believed that:

- Growth decreased because **chemicals in the water affected the processes of transcription and translation.**
- Non-pathogenic bacteria acquired pathogenic traits because **gene cassettes in bacteria were "turned on" as defense mechanisms against the compounds in the water.**
- Real-world conditions created antibiotic resistance because **mutated pathogens could transfer their genes to harmless bacteria through horizontal gene transfer.**

Introduction

- 30-90% of pharmaceuticals that humans and animals take are excreted. Pathogens and pharmaceuticals pass through treatment plants then enter our freshwater systems.
- Few studies have looked at river water alone; it could act as a selective environment for bacteria with pathogenic traits.
- **Bacteria -** prokaryotic organisms. *S. aureus* and *E. coli* are non-pathogenic bacteria.
- **Ohio River -** 900 miles long, containing **treated** and **untreated sewage**.
- **Selective Environment -** environments with conditions that have changed to favor bacteria with specific genes turned on.

Purpose

- General purpose: determine if the Ohio River acts as a selective environment for pathogenic bacteria. This study specifically examines if:
- Growth of *Escherichia coli* and *Staphylococcus aureus* is impacted.
- Ohio River water media encourages the development of pathogenic characterisitcs: antibiotic resistance and larger biofilm formation.
- Real-world conditions of Ohio River water alone create any patterns with antibiotic resistance.

Hypotheses

- **The growth of non-pathogenic *S. aureus* and *E. coli* will be decreased in the presence of Ohio River water media.** Energy normally used for reproducing could be diverted from growth to fighting chemical compounds present in the river that have been proven to damage bacteria.
- **The non-pathogenic bacteria will gain pathogenic traits of antibiotic resistance and larger biofilm formation in the presence of Ohio River water.** Chemicals that come from trash in the water have been shown to create pathogenic characteristics in bacteria that use pollution for food.
- **The real-world conditions of the Ohio River water (not supplemented with media/nutrients) will allow the bacteria to create antibiotic resistance.** Research shows that horizontal gene transfer allows pathogens to pass their resistant genes to non-pathogenic bacteria.

Materials/Methods

The main materials used in this project were:

- Tryptic Soy Broth (media)
- Stock cultures of *S. aureus* and *E. coli*
- Ciprofloxacin and Chloramphenicol antibiotics

12 Hour Growth Curve

- Bacteria were exposed to control or Ohio River water meda over a 12 hour period; growth measured every 20 minutes.

Anitbiotic Resistance Test

- Each bacterium was exposed to Ohio River water (or media) or control media then spread on agar plates. Antibiotic disks (of Ciprofloxacin and Chloramphenicol) added to each plate; plates incubated 12 hours; then zones of inhibition measured.

Biofilm Test

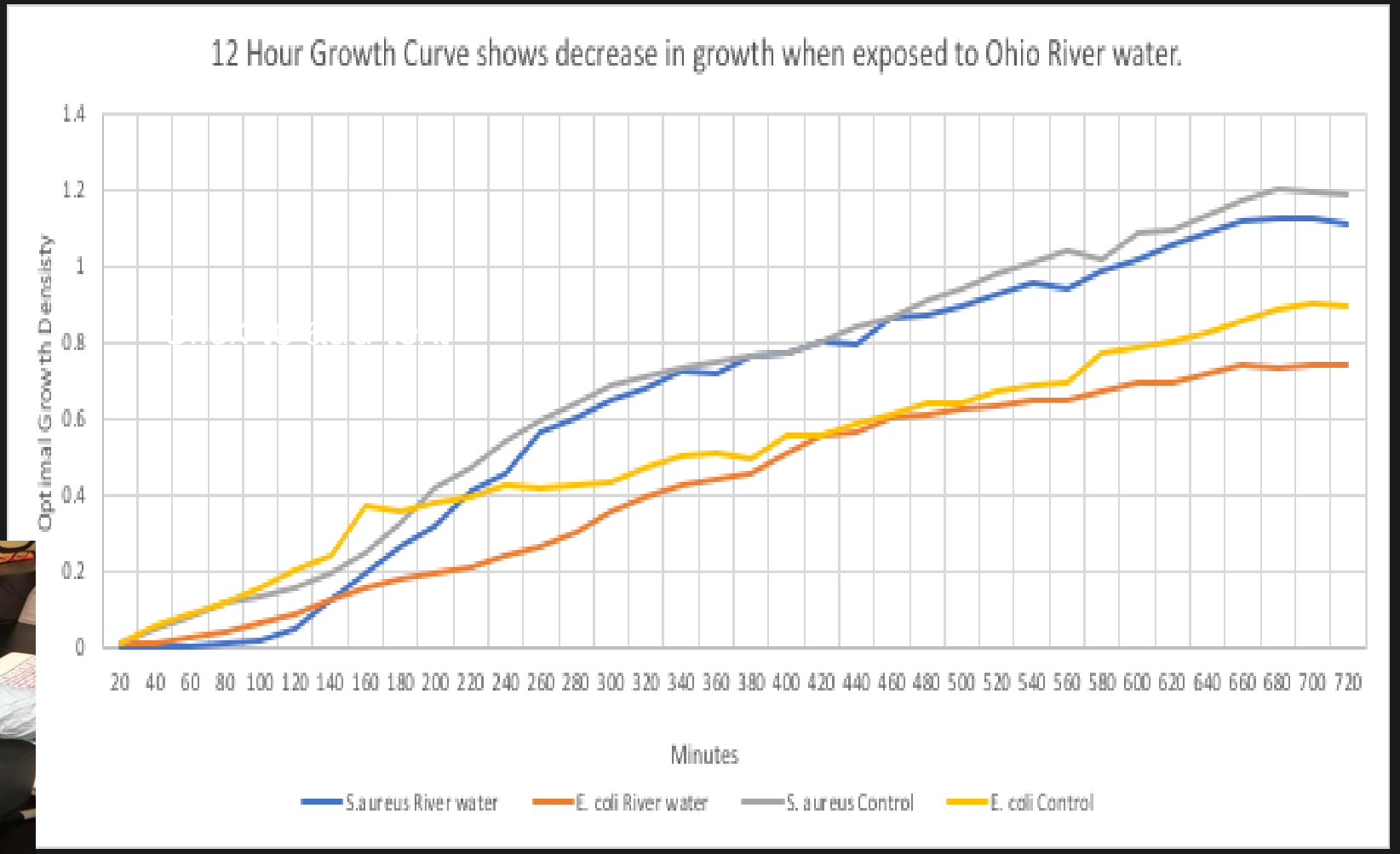
- Bacteria were exposed to either Ohio River water media or control media in tubes, grown for 24 hours, then stained with solution. Biofilm formation indexes measured.

12 Hour Growth Curve

Growth decreases following exposure to Ohio River water media:

- *S. aureus* - 6.08% decrease
- *E. coli* - 17.3% decrease
- Ohio river water acted in a bacteriostatic fashion

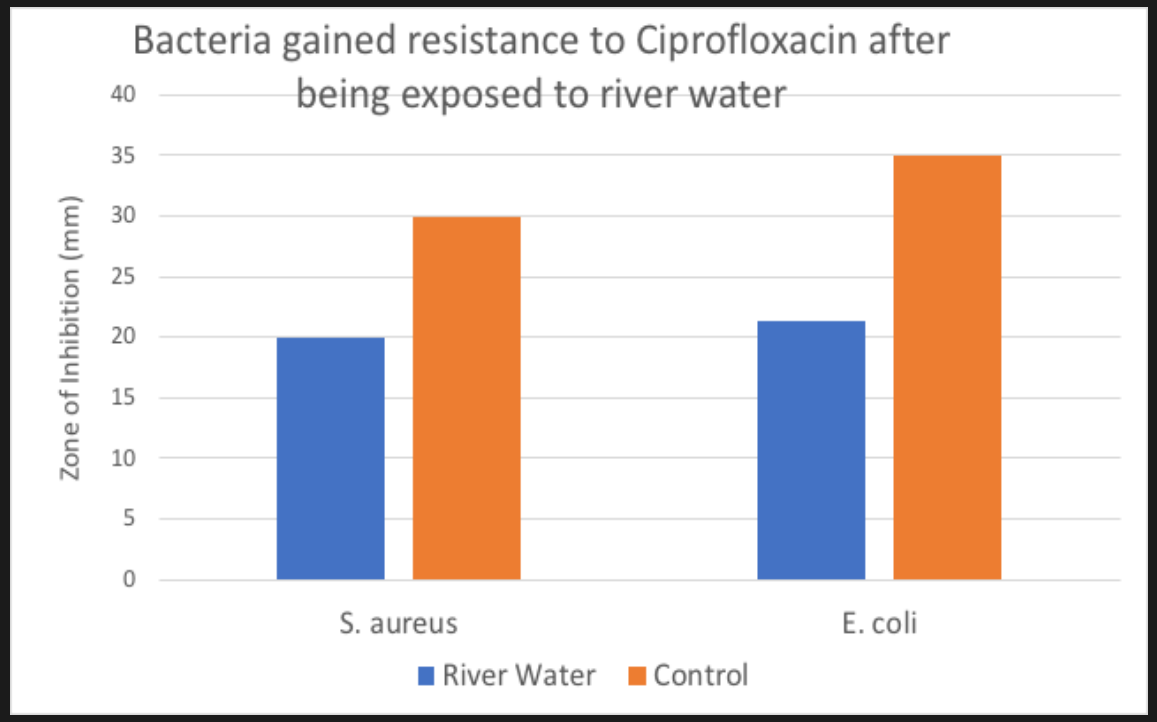
Picture taken by student



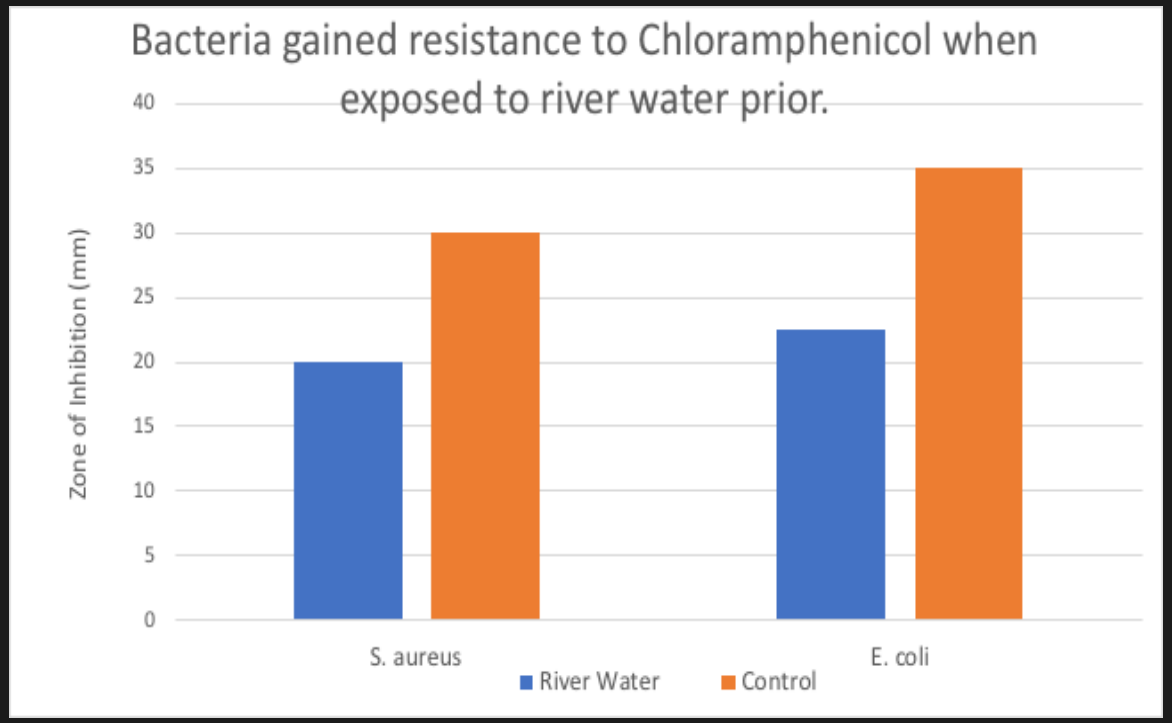
Antibiotic Resistance Test

Picture taken by student

Ciprofloxacin resistance after exposure to Ohio River water media:
S. aureus - 33.3% smaller zone of inhibition
E. coli - 39.14% smaller zone of inhibition



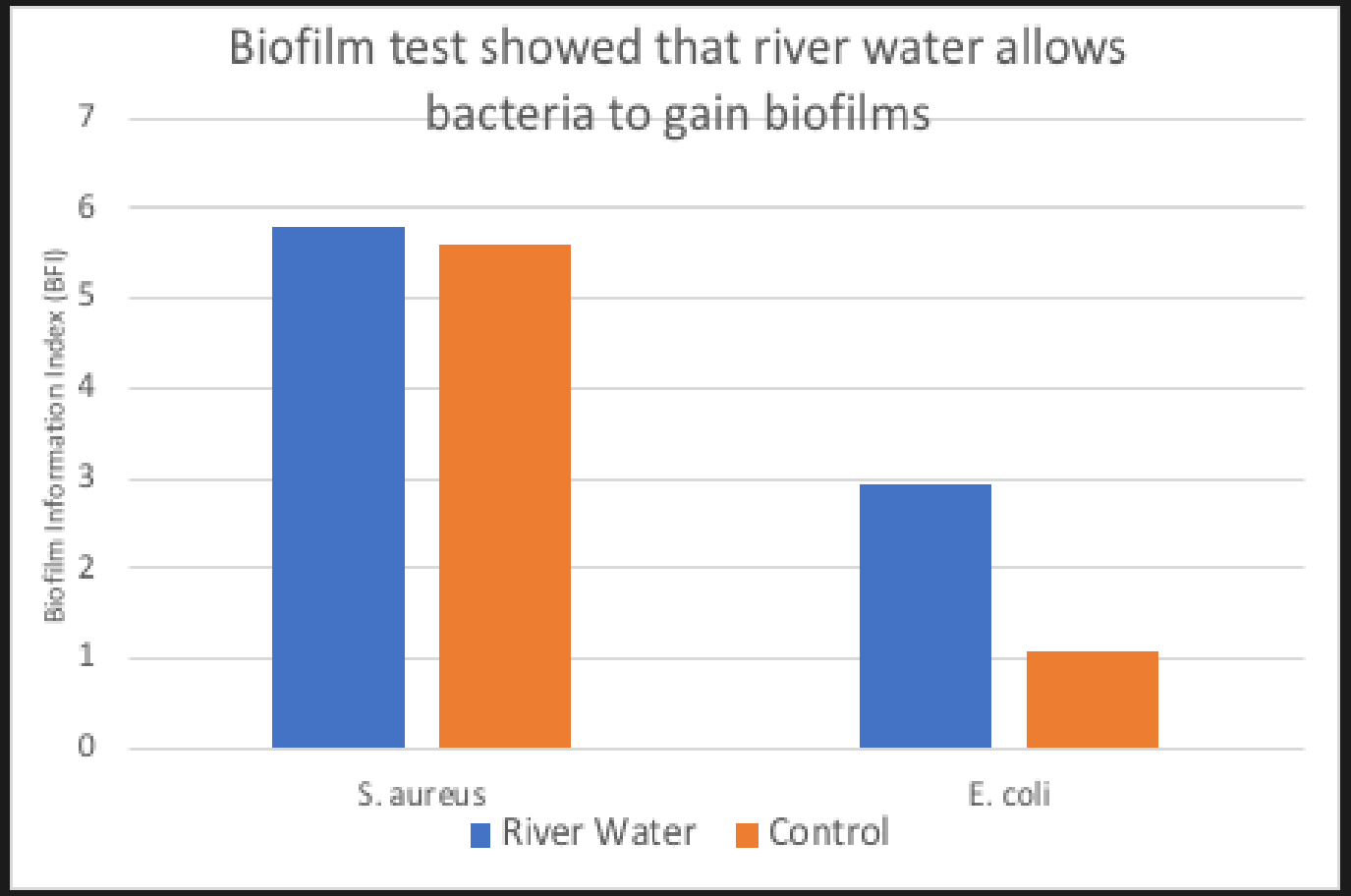
Chloramphenicol resistance after exposure to Ohio River water media:
S. aureus - 33.3% smaller zone of inhibition
E. coli - 36% smaller zone of inhibition



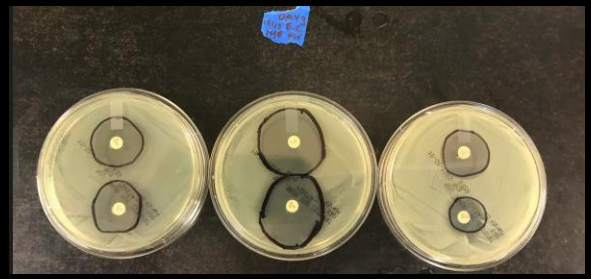
Biofilm test

Biofilm formation index increases following exposure to Ohio River water media:

- *S. aureus* - 3.72% increase
- *E. coli* - 187% increase

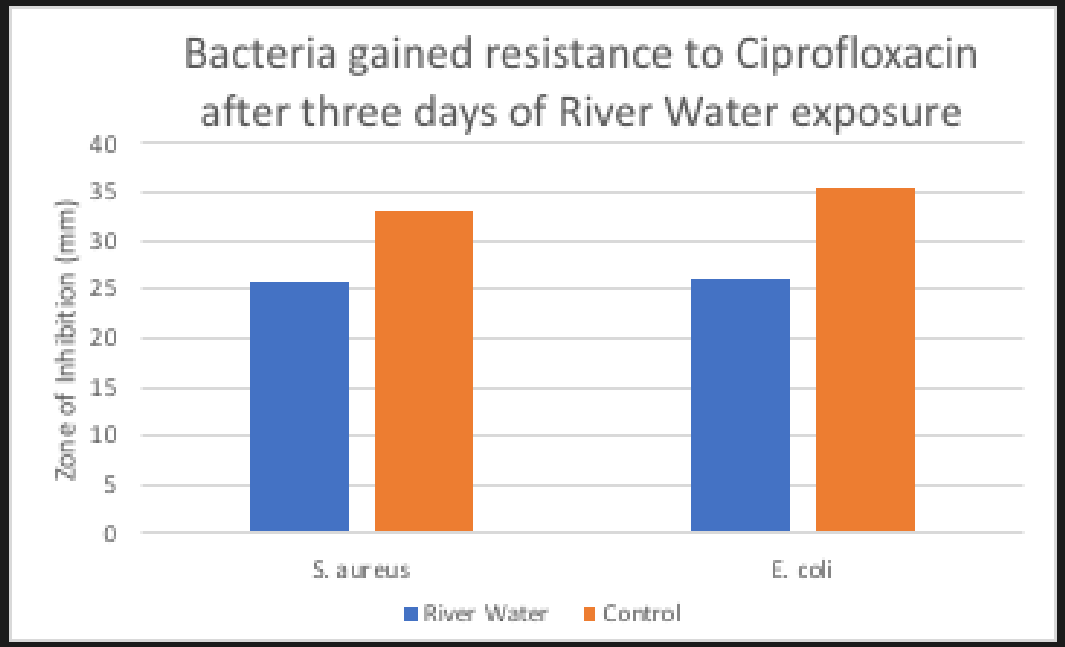


Antibiotic Resistance Test 2

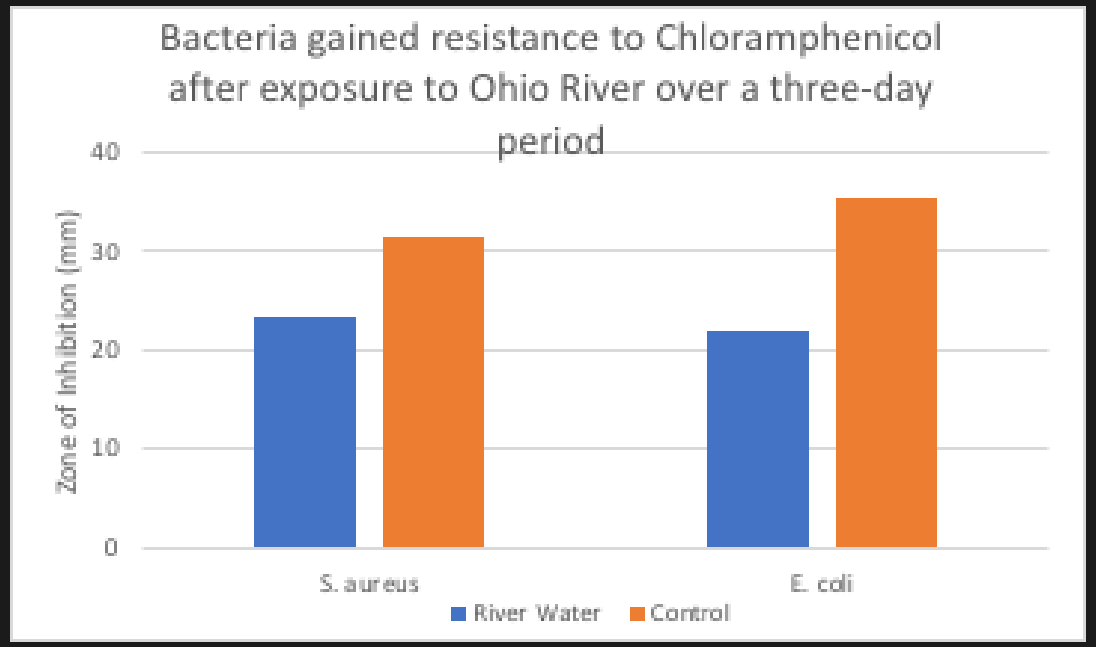


Picture taken by student

Ciprofloxacin resistance after exposure to Ohio River water:
S. aureus - 21.8% smaller zone of inhibition
E. coli - 26.06% smaller zone of inhibition



Chloramphenicol resistance after exposure to Ohio River water:
S. aureus - 25.4% smaller zone of inhibition
E. coli - 38.7% smaller zone of inhibition



Conclusion/Discussion

All hypotheses accepted:

- **Growth of non-pathogenic bacteria decreased** when exposed to Ohio River water media: *S. Aureus* – 6.08% decrease and *E. coli* - 17.3% decrease in growth.
- **Non-pathogenic bacteria created pathogenic traits in the presence of Ohio River water media.** Antibiotic resistance proven by decreased zones of inhibition than control: *S. aureus*—33.3% smaller (Cipro and Chloramphenicol); and *E. coli*—39.14% smaller (Cipro); 36% smaller (Chloramphenicol). Biofilm formation increased: *S. aureus* ---3.72% larger BFI; *E. Coli*---187% larger BFI.
- **The real-world conditions of bacteria exposed to Ohio River water over three days led to resistance**: *S. aureus*—21.8% decrease (Cipro); 25.4% decrease (Chloramphenicol); *E. coli*—26.06% decrease (Cipro); 38.7% decrease (Chloramphenicol).

A challenge:

- Duplicating a real-world enviroment for assessing bacteria in Ohio River water over seven days. Bacteria in tubes lack a constantly changing freshwater supply in the river, which supplies nutrients and removes wastes.

There are several reasons that Ohio River water impacted the bacteria in an antimicrobial fashion:

Growth Decreased	Antibiotic Resistance/Biofilm	Antbiotic Resistance with real world conditions (river water)
• Pharmaceuticals in the water can affect transcription/translation, or the membrane/osmotic pressure.	• Pathogenic traits were on a cassette, which was turned on as a defense mechanism.	• Horizontal gene transfer allowed pathogenic traits to be passed on, creating a selective environment.

Real World Connections/Next Steps

Real World Connections

- 1/3 of drugs prescribed enter wastewater systems, helping to create antibiotic-resistant bacteria.
- More pathogenic water sources require more chemicals to clean water for drinking.
  - Increases risk for "disinfection byproducts" (unintended negative consequences)
  - Better purification costs more – not every community can afford

Next Steps

- Collect samples from other locations along the Ohio and other water sources, identifying highest-risk areas.
- Explore the motility of bacteria in the presence of Ohio River water. If increased, then pathogenicity is spreading more easily, creating an even stronger increased selective environment.

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