



Evaluation of Antibiotic Resistance in Bacteria of White River Waterways: Kirby-Bauer Assays (#2)

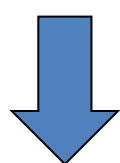
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Background/Introduction:

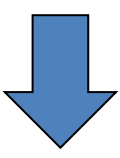
Antibiotic resistance is not only a major threat to public health, with more than 700,000 deaths per year, but also has serious economic impacts.¹ Nosocomial-acquired antibiotic-resistant infections have well-established patterns, while community-acquired antibiotic-resistant infections are relatively unpredictable and continue to increase annually, throughout the United States. A major source of concern includes local waterways and water supply.¹⁻³ The White River water system is a dominant waterway in Indianapolis, supplying drinking water to most of the city, and providing an entry point to sampling community bacteria, such as in the Nina Mason Pulliam EcoLab. In order to assess the level of antibiotic resistance present within this environment, purified bacterial isolates were analyzed with 18 commonly utilized clinical antibiotics.

Methods:

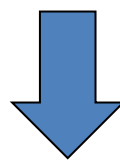
54 bacterial isolates streaked on Mueller-Hinton agar plates, incubated, then inoculated in Mueller-Hinton broth



Tubes grown at 37° C for ~4 hours



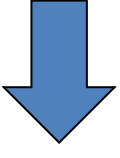
Growth of the inoculated samples measured with UV-Vis. spectrophotometer to ensure appropriate dilutions



Each sample streaked on Mueller-Hinton agar plates, stamped with 18 different antibiotics using a sensi-disc antibiotic disk dispenser & incubated at 37° C



Antibiotic resistance assessed through measurement to the nearest mm of each zone of inhibition



Samples compared to ATCC *E.coli* control strain



Figure 1: Nina Mason Pulliam EcoLab – Bacterial Sample Water Source

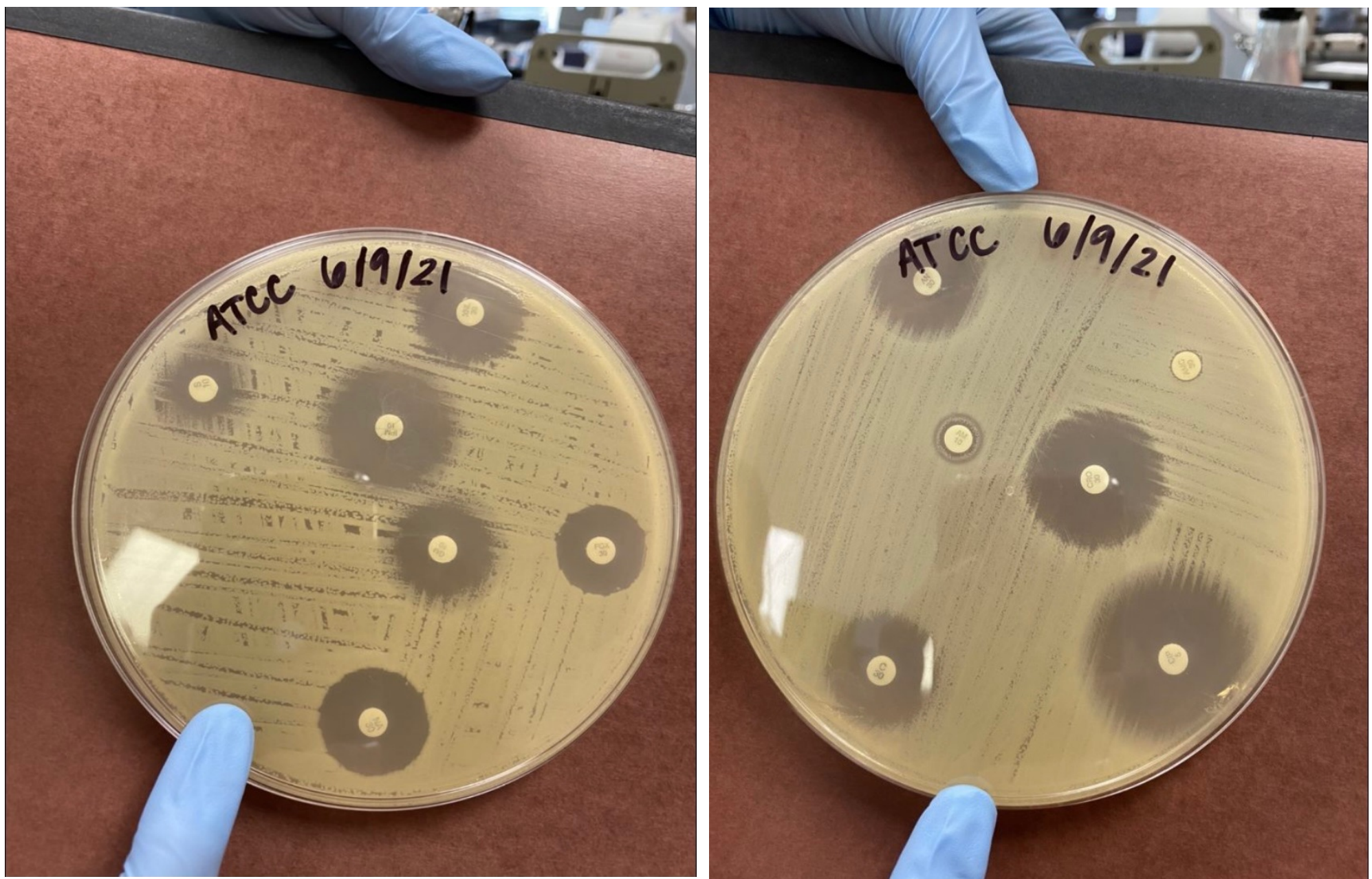


Figure 2: Kirby-Bauer Assays of ATCC *E.coli* control

Results:

| Multidrug Resistance | Aminoglycosides | Aminopenicillins | 1st-Gen Cephalosporins | Fluoroquinolones | Carbapenems | Sulfonamides | Tetracyclines | Glycopeptides |
|----------------------|-----------------|------------------|------------------------|------------------|-------------|--------------|---------------|---------------|
| 54 (100%) | 5 (9%) | 31 (57%) | 32 (59%) | 1 (1%) | 29 (53%) | 12 (22%) | 9 (16%) | 42 (77%) |

Table 1: Percentage of Antibiotic Resistance Displayed Against Antibiotic Classes

- Out of 54 bacterial isolates assayed, all samples demonstrated multidrug resistance.
- Significant multi-strain resistance included resistance against glycopeptide, 1st-, 2nd-, and 3rd-generation cephalosporin, aminopenicillins, and carbapenem antibiotic classes.
- Consistent antibiotic sensitivity against fluoroquinolones, aminoglycosides, and tetracycline antibiotic classes were also displayed.
- Bacterial isolates demonstrated greatest resistance against vancomycin (77%).

Conclusion/Discussion:

- Isolated bacterial samples from the Nina Mason Pulliam EcoLab, which is a part of the White River water system, demonstrates a significant degree of multidrug resistance.
- Further research into the potential sources contributing to the degree of antibiotic resistance present, are needed in facilitating guidance on the proper implementation of public health and environmental health guidelines for control of aquatic ecosystem pollution, such as antibiotic wastes and potential drinking water contamination.
- There are also further implications for changes in antibiotic prescription habits, utilization, and waste management.

References:

1. Manaia CM, Graham D., Topp E., Martinez JL., Collignon P., Gaze WH. (2020) Antibiotic Resistance in the Environment: *Expert Perspectives*. In: Manaia C., Donner E., Vaz-Moreira I., Hong P. (eds) *Antibiotic Resistance in the Environment. The Handbook of Environmental Chemistry*, vol 91. Springer, Cham. https://doi.org/10.1007/978-93-9041-202-4_72
2. Ash RJ., Mauck B, Morgan M. Antibiotic resistance of gram-negative bacteria in rivers, United States. *Emerg Infect Dis*. 2002;8(7):713-716. doi:10.3201/eid0807.010264
3. Sanganyado E, Gwenzi W. Antibiotic resistance in drinking water systems: Occurrence, removal, and human health risks. *Sci Total Environ*. 2019;669:785-797. doi:10.1016/j.scitotenv.2019.03.162