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Survey of Human Impact on Antibiotic Resistances in the Reservoir Dwelling Microbes of Central Arizona

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Introduction

Background: Antimicrobial resistance (AMR) presents a critical threat to animal, environmental, and human health and is responsible for more than one million deaths globally in 2019 alone (WHO, 2023). Environmental surveying of AMR is paramount for action. This work acts as a survey on AMR seen across five freshwater reservoirs of the Central Arizona Highlands. Host to year-round human activity, the lakes present risk of AMR infection to humans and wildlife alike. There are no pre-existing surveys or research of this kind in the region.

Questions: We questioned the influence of proximity to the wastewater treatment plant (WWTP), seasonal rainfall, and whether AMR levels would ↑, ↓, or remain the same upstream vs downstream.

Hypotheses: We hypothesized that samples collected downstream, post-monsoon, and closest to the WWTP (Lake 4) would show reduced levels of susceptibility.

Methods

• Samples sites selected with criteria of being easily accessible to humans and wildlife.
  • Docks, boat ramps, beaches, and shoreline areas with trail access. Each reservoir contained two sampling locations observing these criteria. Sample site (A) located towards each reservoir's inlet and the second site (B) closer to each outlet.

- Post-monsoon samples collected beginning in October 2024, and pre-monsoon season samples being collected the following May 2025.
- Collection used sterile 50 mL cups performed with non-sterile gloves disinfected with 95% ETOH.
- Each sample divided into five 15 mL conical tubes. Conical tubes were then centrifuged.
- Bacterial pellets collected and transferred into TSB. Mixed cultures enriched at 37 C for 24 hours prior to Kirby-Bauer (KB) Assay plating (Jalil et al., 2022).

•Each assay utilized eight antibiotics common to both human and veterinary medicine representing four different mechanisms of action. These included:
  •trimethoprim/sulfamethoxazole (SXT, 25 µg)
  •gentamycin (GEN, 10 µg)
  •tetracycline (TET, 30 µg)
  •ciprofloxacin (CIP, 5 µg)
  •cefazolin (CFZ, 30 µg)
  •bacitracin (BAC)
  •nitrofurantoin (NFT, 300 µg)
  •β-lactam - ampicillin (AMP, 10 µg) in post-monsoon assays and penicillin (PCN, 10 IU) in pre-monsoon assays

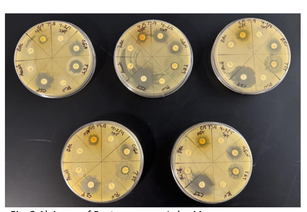


Fig. 3.1) Assay of Post-monsoon Lake 4A

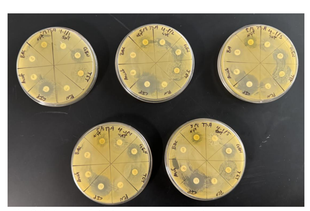


Fig. 3.2) Assay of Post-monsoon Lake 4B

Data

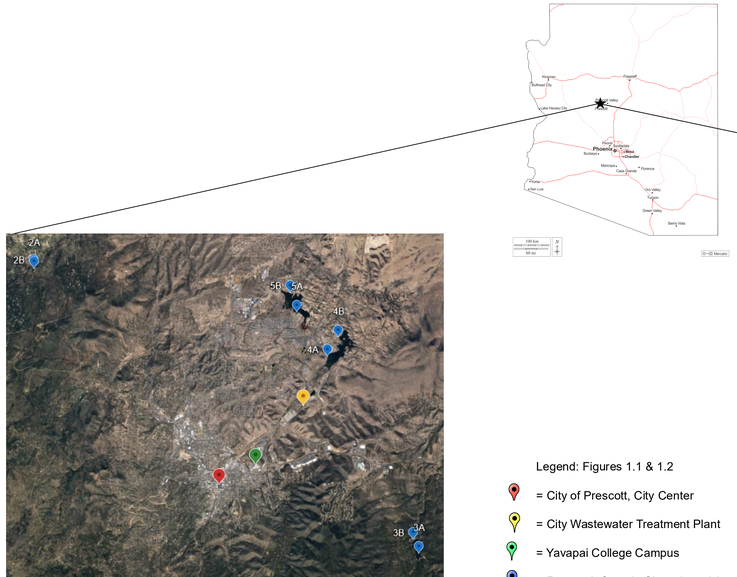


Fig. 1.1) Map of Arizona, zoom on Prescott pre-monsoon sample sites. Lakes 1-5 where A is upstream and B is downstream

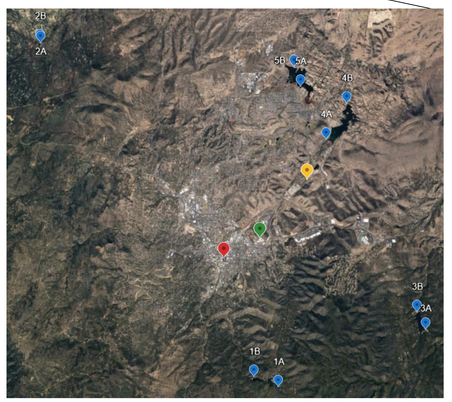


Fig. 1.2) Post-monsoon sample sites. Lakes 1-5 where A is upstream and B is downstream.

Fig. 2.1)

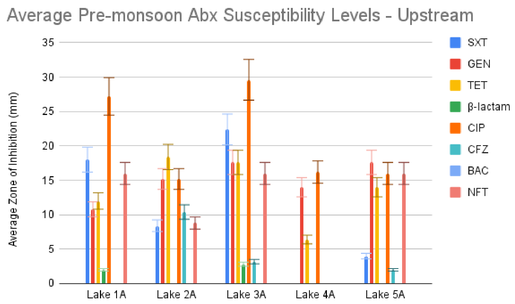


Fig. 2.2)

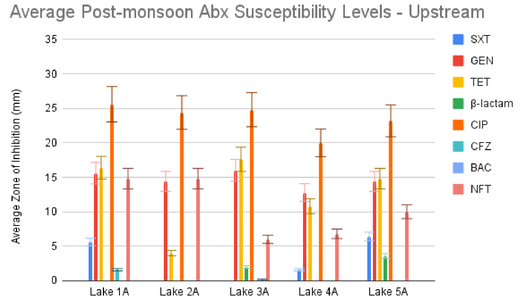


Fig. 2.3)

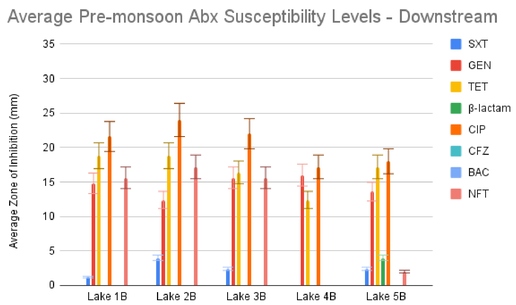
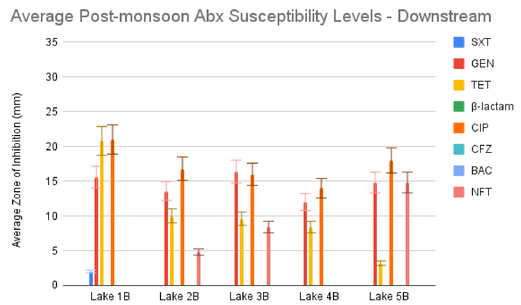


Fig. 2.4)



Figs. 2.1-2.4) Illustrating average zones of antibiotic growth inhibition in mm. For all figures, larger Abx bar Y values (zones of inhibition in mm) and greater quantities of Abx bars X values per reservoir illustrate higher effectiveness of Abx in the sample. Pre-monsoon surface water samples collected in A and C were performed in early October 2024, while post-monsoon samples illustrated in B and D were collected in May of 2025. Data from A-D shows generally greater levels of Abx effectiveness upstream and in pre-monsoon samples both in level of inhibition and in diversity of Abx used. Downstream and post monsoon samples show reduced Abx effectiveness, particularly in Lake 4, having a near direct effluent connection to the city WWTP.

Results/Conclusion

•Lake 4, being the closest to the WWTP displayed the greatest level of AMR of any sample site. Notably with SXT and NFT (commonly used in treatment of UTIs).

•The trend of low susceptibility for CFZ, β-lactam, and BAC was seen in almost every sample site, while CIP consistently inhibited the most growth.

• The same or higher AMR levels were detected downstream in majority of Abx types and locations. Previous findings show decay/dilution of AMR factors downstream over 2-2.5 km (Lee et al., 2021). All downstream sampling sites are < 1.6 km away from upstream counterparts. However, post-monsoon 4B is 3.2 km from WWTP effluent source. May indicate other factors lending to AMR at play.

•Generally, higher AMR levels were detected in post-monsoon samples, lending to the influences of rainfall and short-term flooding in the transport of AMR genes (Mohanta & Goel, 2014).

•The level of AMR seen across these 5 rural reservoirs is alarmingly high, and greater than anticipated.

Future Questions/Exploration

• Repeat seasonal sample collection and testing utilizing improved 0.45 µm filter protocol over centrifugation (Wang et al. 2013).

•PCR sequencing with gene specific primers to identify AMR genes at play, particularly in Lake 4.

•Additional examination of factors that may contribute to high rates of AMR in post-monsoon 4B cultures.

•Isolation of specific microbes, with emphasis on human pathogens like *E. coli* and *S. pyogenes*, for PCR sequencing and KB assays.

•Direct sampling of WWTP effluent.

•Further exploration into local factors influencing a higher downstream AMR levels.

•A mapping of AMR genes in local reservoirs to be shared with hospitals, allowing for better treatment of illness/injury sustained by outdoor hobbyists.

•Exploration of impact of migratory birds, local waterfowl, and agriculture on the spread of AMR genes throughout local water networks.

References

Jalil, A., Gul, S., Bhatti, M. F., Siddiqui, M. F., & Adnan, F. (2022). *High occurrence of multidrug-resistant Escherichia coli strains in bovine fecal samples from healthy cows serves as rich reservoir for AMR transmission*. *Antibiotics, 12*(1), 37. https://doi.org/10.3390/antibiotics12010037

Lee, J., Ju, F., Maile-Moskowitz, A., Beck, K., Maccagnan, A., McArdell, C. S., Dal Molin, M., Fenicia, F., Vikesland, P. J., Pruden, A., Stamm, C., & Bürgmann, H. (2021). Unraveling the riverine antibiotic resistome: The downstream fate of anthropogenic inputs. *Water Research, 197*, 117050. https://doi.org/10.1016/j.watres.2021.117050

Mohanta, T., & Goel, S. (2014). Prevalence of antibiotic-resistant bacteria in three different aquatic environments over three seasons. *Environmental Monitoring and Assessment, 186*(8), 5089–5100. https://doi.org/10.1007/s10661-014-3762-1

Wang, C., Gu, X., Zhang, S., Wang, P., Guo, C., Gu, J., & Hou, J. (2013). Characterization of antibiotic-resistance genes in antibiotic-resistant *Escherichia coli* isolates from a lake. *Archives of Environmental Contamination and Toxicology, 65*(4), 635–641. https://doi.org/10.1007/s00244-013-9932-2

World Health Organization. (2023, December 15). *Antimicrobial resistance*. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance