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# The Investigation of Antibiotic Resistance in Tangxun Lake

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***Abstract:*** *The investigation of antibiotic resistance in Tangxun Lake explores the consequences of continuous antibiotic discharge into the water. The study focuses on the potential impact of human activities on antibiotic discharges, with a specific emphasis on antibiotic resistance in lake bacteria. The method involved sampling from three locations within the lake, employing Tryptic Soy Agar as the culture medium. Antibiotic treatments with penicillin and cephalosporin were applied, followed by incubation and subsequent culture on Plate Count Agar. Results indicate antibiotic resistance in lake bacteria, with variations in sensitivity to penicillin and cephalosporin across locations. Graphical representations illustrate significant differences in bacterial growth with antibiotic exposure. The study highlights the continued discharge of antibiotics into Tangxun Lake.*

***Keywords:*** *Freshwater, antibiotic, antibiotic resistance, penicillin, cephalosporin, Tangxun Lake*

## 1. Introduction

Fresh water makes up only 0.01% of the World's water and approximately 0.8% of the Earth's surface, yet this tiny fraction of global water supports at least 100000 species out of approximately 1.8 million – almost 6% of all described species. Freshwaters are experiencing declines in biodiversity far greater than those in the most affected terrestrial ecosystems, and if trends in human demands for water remain unaltered and species losses continue at current rates, the opportunity to conserve much of the remaining biodiversity in freshwater will vanish before the 'Water for Life' decade ends in 2015 [1].

The discovery and development of antibiotics have significantly impacted public health, transforming medicine and surgery [2]. However, overuse and misuse of antibiotics have led to the emergence of antibiotic-resistant strains of bacteria, posing a growing global health threat. Genetic mutation occurs when the bacteria's DNA, or genetic material, randomly changes. If these changes let the bacteria evade an antibiotic that would have otherwise killed it, it will be able to survive and pass on this resistance when it reproduces. Over time, the proportion of resistant bacteria will increase as nonresistant bacteria are killed by the antibiotic. Eventually, the drug will no longer work on these bacteria because they all have the mutation for resistance [3].

Tangxun Lake, once Wuhan's largest pristine lake with a subtropical humid monsoon climate, spans Jiangxia District, Hongshan District, and Donghu Hi-Tech Development Zone. It covers 47.62 square kilometers, with the inner Tangxun Lake constituting about one-third and the outer Tangxun Lake about two-thirds. The lake has a 122.8-kilometer shoreline, an average depth of 2.32 meters, and plays a crucial role as a plains water network in the middle and lower reaches of the Yangtze River, aiding flood resistance and runoff regulation. It serves as a backup water source for Wuhan [4].

However, recent years have seen increased population density, industrial park construction, and aquaculture development, leading to severe water pollution from sewage treatment plant effluents and mixed sewage. Aquatic habitat deterioration, exacerbated by reduced aquatic replenishment, and the destruction of aquatic plants due to previous aquaculture use have resulted in a more critical water environmental problem at Tangxun Lake. Additionally, urban development has contributed to declining water quality, surpassing standards in recent years [5].

The urbanization of the lakefront area is just within this decade. Before the completion of the urban sewer system, most sanitary sewage is directly discharged into the lake. This may continually expose the bacteria to antibiotics, resulting in antibiotic resistance. The expansive lake area encompasses regions with varying human activities, allowing us to create environments with and without antibiotic exposure. Our study aims to explore if there is continued antibiotic discharges in Tangxun Lake.

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## 2. Method

We took lake samples from three locations on 13th Oct 2023. Location A is within an angling area while location B and C are within a forest part. The information of the three locations in included in table 1.

*Table 1: Information of Sampling Location*

| Location | Latitude (N) | Longitude (W) | pH | TDS |
|---|---|---|---|---|
| A | 30.453520 | 114.340457 | 8.5 | 159 |
| B | 30.413580 | 114.325145 | 8.91 | 171 |
| C | 30.402850 | 114.420845 | 7.51 | 102 |

Upon our return to the laboratory, we opted for Tryptic Soy Agar as the designated culture medium. The lake samples from each location were divided into three segments: one served as a blank control, while the other two underwent treatment with penicillin and cephalosporin, respectively. The information of all the treatments is included in table 2. Subsequently, these samples were placed in petri dishes and introduced into an incubator. Following a week of incubation, Plate Count Agar was employed as the new culture medium. The bacteria in the petri dishes were appropriately diluted and transferred to the fresh medium. Our final step involved enumerating the bacterial colonies on each plate. A notable challenge encountered during this experiment was the inadvertent contamination of the samples, hindering our ability to accurately identify and count the bacteria at the conclusion of the study. Unfortunately, petri dishes from location C exhibited fungal growth, rendering the entire dish unsuitable for further analysis and prompting its abandonment.

*Table 2: Information of all the Treatment*

| Location | Treatment | | |
|---|---|---|---|
| | Water | Penicillin | Cephalosporin |
| A | AX | AY | AZ |
| B | BX | BY | BZ |
| C | CX | CY | CZ |

We photograph each petri dish with bacteria growing in it and count the percentage area that the bacteria occupied. We then apply t-test or ANOVA to analyze if there is significant difference in the percentage area that bacteria grow in difference groups.

## 3. Result

The bacteria in both locations have shown antibiotic resistance to some extent. In location A, bacteria are more sensitive to cephalosporin compared with penicillin. For location A, significant difference is found between each pair of the groups (figure 1).

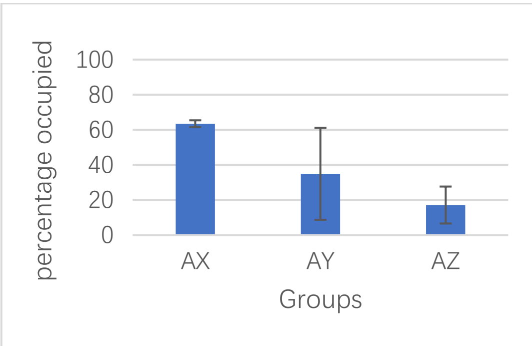


*Figure 1: The comparison of lake sample in location A growing in culture medium without antibiotic (AX), treated with penicillin (AY) and cephalosporin (AZ)*

For location B, bacteria have developed resistance to cephalosporin but they remain extremely sensitive to penicillin. For location B, significant difference is found between BX and BY; BY and BZ (figure 2).

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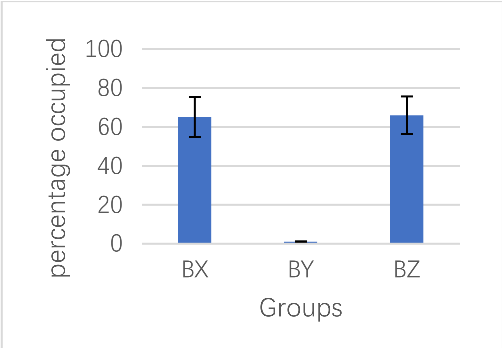


*Figure 2: The sample in location B growing in culture medium without antibiotics (BX), treated with Penicillin (BY) and cephalosporin (BZ).*

Our result suggests that the bacteria in Tangxun Lake has actually been exposed to the antibiotics we had tested. There is no significant difference in bacteria number between the treatments without bacteria (figure 3).

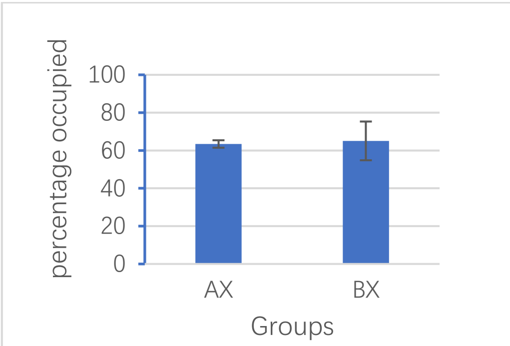


*Figure 3: Comparison of samples without being treated with antibiotics*

The bacteria in different location may have experienced exposure to different antibiotics. The number of bacteria in location B sample is significantly smaller that in location A when treated with penicillin (figure 4).

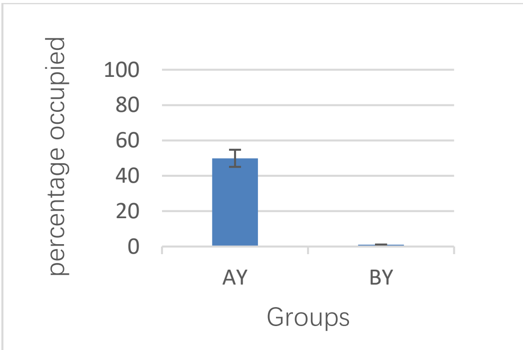


*Figure 4: Comparison of samples being treated with penicillin*

The number of bacteria in location A sample is significantly smaller that in location B when treated with cephalosporin (figure 5).

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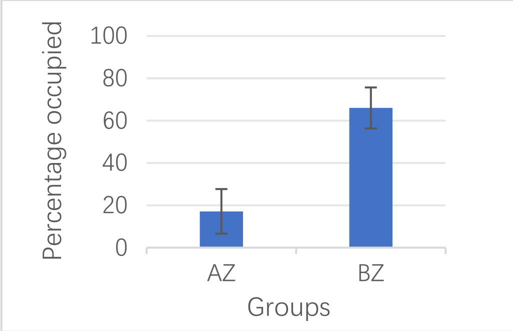


*Figure 5: Comparison of samples being treated with cephalosporin*

All the evidence suggests that there is continued antibiotic discharge into Tangxun Lake.

## 4. Conclusion

Continuous discharge of antibiotics into rivers and lakes can have significant and far-reaching consequences on both aquatic ecosystems and human health. Prolonged exposure to antibiotics in water bodies can lead to the development of antibiotic-resistant bacteria. The constant presence of these drugs exerts selective pressure on bacteria, favoring the survival and proliferation of resistant strains. This can contribute to the spread of antibiotic resistance in the environment [6].

Moreover, contaminated water sources can pose risks to human health. If people consume water or consume aquatic organisms that have been exposed to antibiotics, there is a potential for the development of antibiotic resistance in human pathogens. Additionally, antibiotic residues in water may contribute to the overall burden of antibiotic exposure for communities relying on these water sources [7]. Besides, antibiotics introduced into water bodies may undergo various chemical and biological transformations. These transformed compounds, known as metabolites, can persist in the environment and may have different ecological and toxicological properties compared to the original antibiotics.

Considering the serious consequence of antibiotic resistance, we propose that further researches should be carried out to identify the sources of antibiotic discharge, so as to prevent the spread of antibiotic resistance.

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