Comparative Genomics to Investigate the Resistome and Bacterial Heterogeneity in the Creekside Ecosystem in Navotas City: An Exploratory Research

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ABSTRACT

Antimicrobial resistance (AMR) is an escalating global health threat that reduces the effectiveness of antimicrobial medications, leading to higher risks of infection, longer durations of illness, and increased mortality. In the Philippines, poor wastewater management contributes to the spread of resistant microorganisms, particularly in Navotas City—an urban area along the polluted Navotas-Malabon-Tullahan River system. This study aimed to investigate the bacterial community and resistome in water samples collected from three creekside locations in Badeo Dos, Navotas City, using shotgun metagenomic sequencing. Specifically, it sought to identify the most abundant bacterial taxa and acquired antimicrobial resistance genes (ARGs) across sampling sites. A descriptive-correlational design was used, with triplicate 600 mL water samples collected during the first and fourth weeks of January 2025. DNA was extracted and validated using Qubit, NanoDrop, and gel electrophoresis, followed by sequencing via Illumina NovaSeq. Taxonomic classification employed QIIME, KRAKEN, and BRACKEN, while ARGs were identified using Q2-RGI and the CARD database. Results showed 86.6% bacterial reads, with 68 genera dominated by *Pseudomonadati* and *Bacillati*. Pathogenic genera such as *Pseudomonas* and *Rhodococcus* were prevalent. ARGs conferring resistance to rifampicin (*rpoB mutants*), sulfonamides (*sul1*), macrolides (*tlrC*), aminoglycosides (*aadA6*), and various beta-lactams including carbapenemases and cephalosporin-resistant genes were detected. No significant differences in bacterial or ARG abundance were observed among sites, indicating uniform distribution likely due to water flow and horizontal gene transfer. The findings confirm that Navotas River is a reservoir for AMR, highlighting the need for regular monitoring, stricter wastewater regulation, and expanded temporal studies to track resistance dynamics.

SITE 1 — Located next to a ship repair center

SITE 2 — Impacted by residential wastewater

SITE 3 — Near the creek's confluence with the Navotas River

OBJECTIVES

GENERAL

To identify the bacterial community and resistome present in the environmental water samples from each of the three sampling locations.

SPECIFIC

- Determine the most abundant bacteria identified in the Creekside at Badeo Dos, Navotas City, using shotgun metagenomic sequencing data
- Identify the most abundant extrinsic ARGs identified in the Creekside at Badeo Dos, Navotas City, using shotgun metagenomic sequencing data.
- Determine if there is any significant difference in the relative abundance of identified bacteria and extrinsic ARGs from the three sampling points of the Creekside at Baseo Dos, Navotas City.

METHODOLOGY

WATER COLLECTION
Collection on the 1st week and last week of January 2025 together with PH & Temperature testing at *Badeo Dos, Navotas City Creekside*

WATER FILTRATION
Three (3) times filtration

GENOMIC DNA EXTRACTION
HiPurA Water DNA Purification Kit

QUALITY CONTROL
Invitrogen Qubit 4 Fluorometer & FLUOstar Omega Multiskan Skyhigh Micriplate Nanodrop Reader

NEXT GENERATION SEQUENCING-SHOTGUN METAGENOMICS
Illumina NOVASeq XPlus Platform at Australian Genome Research Facility in **Melbourne, Australia**

BIOINFORMATICS
QIIME 2, KRAKEN 2, CARD

A descriptive-correlational design was used at three creekside sites with varying human activity. Triplicate water samples (600 mL/site) were collected during the first and fourth weeks of January 2025. DNA was extracted and validated using Qubit, NanoDrop, and gel electrophoresis. Shotgun metagenomic sequencing was performed via Illumina NovaSeq (15 million reads/sample). Taxonomic classification used QIIME, KRAKEN, and BRACKEN, while ARGs were identified through Q2-RGI with the CARD database. One-way ANOVA assessed differences in bacterial and ARG abundance across sites.

INTRODUCTION

Antimicrobial resistance (AMR) is a growing global health crisis marked by microbes' ability to survive antibiotics, leading to hard-to-treat infections and higher mortality. In the Philippines, unregulated antibiotic use, poor sanitation, and inadequate wastewater treatment worsen AMR spread. Urban waterways like the Navotas River are key reservoirs and pathways for resistant bacteria and genes due to heavy human activity. In Navotas City, polluted creekside waters face contamination from residential waste, industrial effluents, and poor sanitation, driving bacterial growth and resistance. This study uses shotgun metagenomics and bioinformatics to assess bacterial diversity and ARG profiles across creekside sites. Understanding the resistome supports targeted environmental and public health interventions to curb AMR in urban waters.
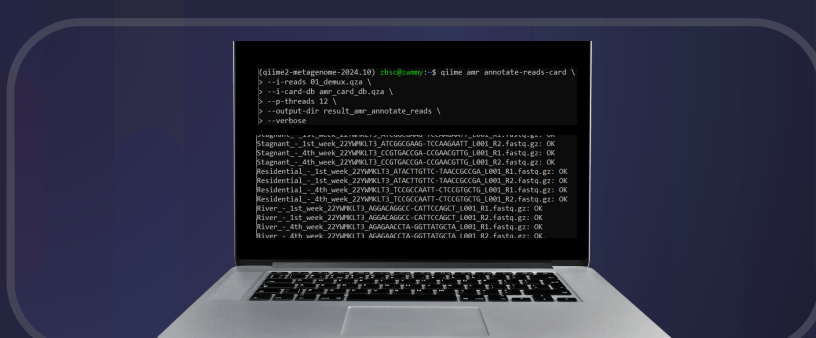
RESULTS AND DISCUSSION

TOP 10 MOST ABUNDANCE OF BACTERIA

The genus *Caulobacter* (15.82%) is the most prevalent bacteria but possesses little clinical relevance. The second prevalent bacteria is *Pseudomonas* (15.73%), a ubiquitous bacteria known for nosocomial infections, and resistance to the elements and antibiotics through acquiring other ARGs such as altering the polymerase β-subunit to obtain resistance to rifampicin. The other bacteria in respect of their abundance are *Parazoarcus* (11.67%), *Rhodococcus* (10.95%), *Alcanivorax* (7.93%), *Streptomyces* (4.42%), *Gimesia* (3.23%), *Sulfitobacter* (2.25%), *Sphingopyxis* (2.09%), and *Alloalcanivorax* (1.76%).
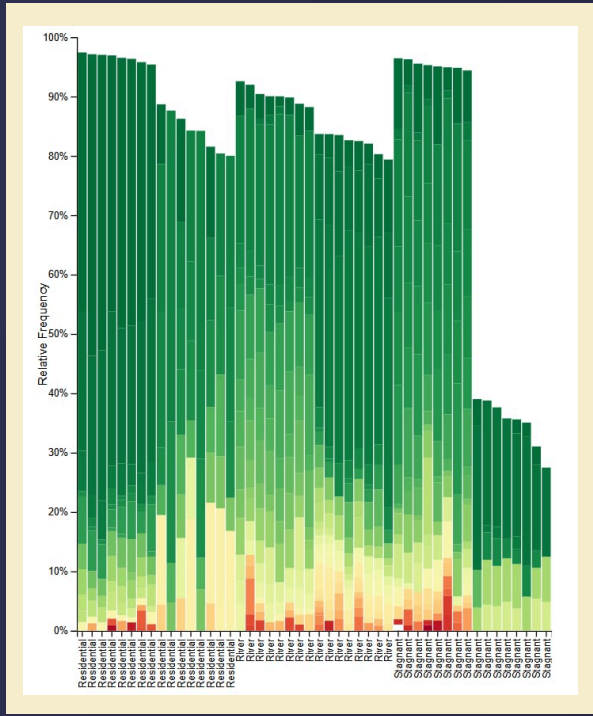
TOP 10 MOST ABUNDANT ANTIBIOTIC RESISTANCE GENES
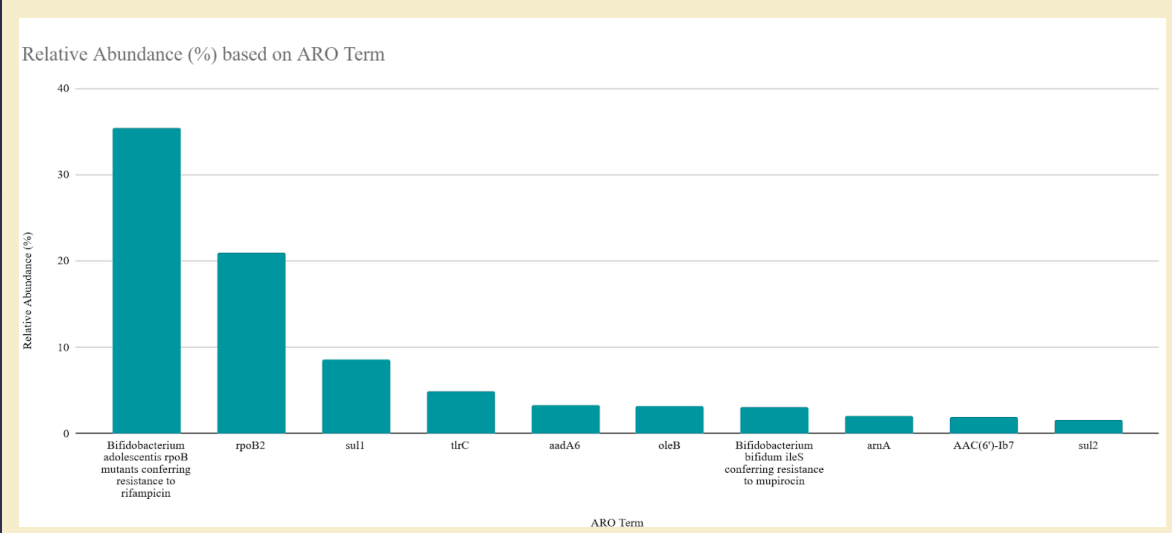
The most abundant resistance gene was the *Bifidobacterium adolescentis rpoB* mutant (35.41%), conferring rifampicin resistance. While *Bifidobacterium* species are generally beneficial, their ability to harbor and transfer ARGs raises concerns about horizontal gene transfer. *rpoB2* (20.94%) was the second most abundant, also linked to rifampicin resistance. Other key ARGs included *sul1* and *sul2* (sulfonamide resistance), *tlrC* and *oleB* (macrolide/lincosamide protection and efflux), *aadA6* and *AAC(6')-Ib7* (aminoglycoside inactivation), *Bifidobacterium bifidum ileS* (mupirocin resistance), and *arnA* (polymyxin resistance).

ANALYSIS OF VARIANCE (ANOVA)

COMPARING BACTERIAL GENUS
There are no statistically significant differences in the mean scores across the three sampling points suggesting that bacteria are consistent in all sampling sites.

Source of Variation	SS	df	MS	F	P-value
Between Groups	0.67	2	0.033	0.003	0.997
Within Groups	305.8	27	11.326		
Total	305.867	29			

COMPARING ARGS
There are no statistically significant differences in the mean scores across the three sampling points suggesting that ARGs are consistent in all sampling sites.

Source of Variation	SS	df	MS	F	P-value
Between Groups	14.6	2	7.3	0.255	0.777
Within Groups	773.4	27	28.644		
Total	788	29			

The physicochemical parameters and sequencing requirements of the extracted DNA were within acceptable ranges. This study showed a diverse microbial community with known pathogenicity, including Pseudomonas, Sulfitobacter, Streptomyces, and Rhodococcus. The rpoB mutant in Bifidobacterium adolescentis (35.41%), conferring rifampicin resistance, was the most prevalent ARG, followed by rpoB2 in Nocardia (20.94%). Additional significant ARGs included sul1 (8.61%) and tlrC (4.90%), which are associated with sulfonamide and macrolide-lincosamide resistance. Variants of B-lactamase genes (VIM, NDM, and OXA) were detected in P. aeruginosa and Klebsiella spp., and genes conferring resistance to aminoglycosides were identified in P. aeruginosa and Serratia marcescens. In addition, some genes with reduced susceptibility to carbapenems including OXA-51-like, OXA-60-like, MSI-OXA family, class D LRA, AIM, metallo-β-lactamases (MBL), the L1 family, and POM were prevalent. Cephalosporin-resistant genes were also identified, including Rm3 family, PDC, ALG6, CAU, SRT, STA, THIN-B, PME, PER, AXC, and AQU. ANA, SIE, and MBL which showed resistance activity against multiple β-lactams. Lastly, statistical analysis showed no significant ARG abundance differences across sites (p = 0.997; p = 0.777).

CONCLUSION

This study confirms the presence of diverse antibiotic resistance genes (ARGs) and resistant bacteria in the Navotas River, a waterway heavily impacted by human activity. Clinically important markers—such as *rpoB* mutations and β-lactamase variants (*blaNDM, blaVIM, OXA* types)—were found in pathogens like *Serratia marcescens, Klebsiella spp.,* and *Pseudomonas aeruginosa*, posing risks to nearby communities lacking clean water and sanitation. Although no significant differences in ARG abundance were found across sites, the widespread presence suggests a broad AMR burden likely driven by horizontal gene transfer and water flow. These findings stress the urgent need for integrated AMR monitoring, environmental regulation, and routine surveillance to detect emerging threats. Without prompt action, environmental reservoirs like the Navotas River could accelerate resistance spread and challenge current treatment strategies.

RECOMMENDATION

1. Future studies can culture identified bacterial species for phenotypic analysis and explore transcriptomics to assess gene expression.

2. Utilizing High-Performance Computing (HPC) and SciCloud can enhance data processing and scalability.

3. Expanding sampling scope and including temporal analysis can provide a more comprehensive view of ARGs and bacterial diversity.

REFERENCES: