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Literature Review: Genomic Profiling for Monitoring Antimicrobial Resistance in Environmental Wastewater

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Abstract

Both human and environmental health are seriously jeopardised by the rise of antimicrobial resistance (AMR). The monitoring of antimicrobial resistance (AMR) in populations has become increasingly dependent on wastewater-based surveillance. One promising comprehensive high-throughput approach to identifying and measuring resistance genes is metagenomics, which entails sequencing and analysing genetic material from environmental samples. Nevertheless, its efficacy is constrained by methodological obstacles such inconsistent bioinformatics, low-abundance gene detection, and environmental heterogeneity. Findings from important research on AMR monitoring using metagenomics in wastewater are synthesised in this review. It emphasises new developments such as coupled qPCR-metagenomic methods for higher sensitivity and specificity, better bioinformatics pipelines, and synthetic DNA standards. Machine learning for pattern identification and worldwide surveillance networks for coordinated monitoring are two options proposed in the study to fill up the gaps in standardisation and data interpretation. In order to make metagenomic AMR monitoring in wastewater settings more accurate and scalable, it is essential to establish uniform procedures and to increase the worldwide surveillance infrastructure.

Keywords: *Antimicrobial resistance, wastewater surveillance, metagenomics, quantitative metagenomics, qPCR, bioinformatics, low-abundance gene detection*

1. Introduction

The rise of antimicrobial resistance (AMR) is a major concern in public and environmental health across the world. The development of resistance to antimicrobial agents by bacteria, viruses, fungi, and parasites is known as antimicrobial resistance (AMR). This phenomenon makes treatments for these illnesses less effective and raises the likelihood of severe infections and disease outbreaks (Miłobedzka et al., 2022). The use of wastewater-based surveillance to track antimicrobial resistance (AMR) in populations has recently become popular (Tiwari et al., 2022). A useful tool for detecting AMR in complicated wastewater matrices is genomic profiling using metagenomics, which includes sequencing genetic material from environmental samples. There are a number of obstacles that metagenomics must overcome before it can reach its full potential (Davis et al., 2025). These include the need to define quantitative boundaries, find genes with low abundance, and guarantee methodological consistency between investigations.

This literature review explores the current state of research in wastewater-based AMR surveillance using genomic profiling techniques, focusing on metagenomics. It synthesizes findings from key studies, identifies methodological gaps, and proposes improvements to enhance the accuracy, scalability, and applicability of metagenomics for environmental AMR monitoring.

2. Current State of Research

2.1 Wastewater-Based AMR Surveillance

Wastewater environments serve as a reservoir and transmission pathway for AMR genes, making them ideal targets for surveillance. Miłobedzka et al. (2022) highlighted that wastewater reflects the health status of a population and serves as a dynamic indicator of AMR trends. Their study emphasized the complexity of wastewater matrices and the need for robust analytical methods to detect resistance genes accurately. Wastewater contains a mixture of human-associated and environmental bacteria, complicating the detection and quantification of resistance genes.

In their comprehensive review of AMR monitoring using wastewater, Tiwari et al. (2022) highlighted the fact that different research used different sampling techniques, sequencing depths, and bioinformatics processes. The authors came to the conclusion that in order to get consistent and comparable findings from various sites and phases of wastewater treatment, methodology standardisation is essential.

When it came to antimicrobial resistance (AMR) monitoring in wastewater, Knight et al. (2024) compared metagenomic methods with high-throughput quantitative PCR (HT qPCR). The results showed that metagenomics has a wider range of detection capabilities, although it is more unpredictable at low abundance levels. It seems that merging qPCR with metagenomics might enhance detection precision and provide supplementary understanding of AMR gene dynamics.

2.2 Metagenomic Techniques for AMR Detection

Quantitative metagenomics involves extracting DNA from environmental samples, sequencing the genetic material, and mapping the sequences to known resistance genes. Davis et al. (2025) introduced a novel quantitative metagenomic approach for wastewater-based AMR surveillance. They demonstrated that metagenomic data could achieve strong linearity in gene detection at concentrations as low as 2×10–3 m/m% ($R^2 > 0.95$). The calculated limits of quantification (LoQ) and detection were 1.3×10^3 and 1 gene copy per µL of DNA extract, respectively. These findings establish a quantitative benchmark for metagenomic surveillance, enhancing the reliability of low-abundance gene detection.

Liguori et al. (2022) proposed a standardized framework for antimicrobial resistance monitoring in water environments. Their study highlighted the importance of quality control measures, such as spiking experiments with reference standards, to validate the accuracy and reproducibility of metagenomic analyses. They also recommended incorporating metadata, including sequencing depth, reference genome coverage, and statistical confidence levels, to strengthen data interpretation.

The worldwide distribution of AMR genes was shown by Munk et al. (2022), who performed a massive genomic study of effluent from 101 nations. Based on their findings, metagenomics has the potential to provide a worldwide picture of the distribution and development of AMR genes. Nevertheless, they did point out that there were discrepancies in gene detection rates and abundance estimations due to the fact that sequencing platforms and bioinformatics pipelines may be somewhat variable.

3. Methodological Approaches

3.1 Comparative Analysis of qPCR and Metagenomics

Knight et al. (2024) compared qPCR and metagenomic approaches for AMR surveillance, finding that qPCR offers higher sensitivity for specific genes but is limited to known targets. In contrast, metagenomics enables the detection of novel and unexpected resistance genes but may struggle with low-abundance gene quantification due to background noise. Combining these methods could provide a more comprehensive understanding of AMR dynamics in wastewater.

3.2 Benchmarking and Validation Strategies

Davis et al. (2025) employed synthetic DNA standards (meta-sequins) to benchmark metagenomic quantification. Their study demonstrated that meta-sequin ladders exhibited strong linearity and minimal GC bias at low input concentrations. This approach provides a reproducible method for validating metagenomic data, establishing confidence in low-abundance gene detection.

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4. Challenges and Limitations

4.1 Low-Abundance Gene Detection

Detecting low-abundance genes remains a major challenge in metagenomics due to background noise and sequencing depth limitations. Davis et al. (2025) showed that the LoQ and detection limits could be improved through higher sequencing depth and optimized bioinformatics pipelines.

4.2 Bioinformatics and Data Interpretation

Munk et al. (2022) highlighted inconsistencies in gene detection rates across different bioinformatics platforms. Developing standardized bioinformatics pipelines and reference databases is essential to improve data consistency and comparability.

4.3 Sampling and Environmental Variability

Miłobedzka et al. (2022) emphasized the influence of environmental factors, such as seasonal variation and wastewater composition, on AMR gene detection. Establishing consistent sampling protocols and adjusting for environmental variability are critical for accurate trend analysis.

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5. Proposed Improvements and Future Directions

5.1 Integration of qPCR and Metagenomics

Combining qPCR and metagenomics could enhance the sensitivity and specificity of AMR detection. qPCR can provide high-confidence quantification of known genes, while metagenomics can identify novel resistance genes and track their evolution.

5.2 Machine Learning for Data Analysis

Machine learning algorithms could enhance bioinformatics analysis by identifying patterns and correlations in large metagenomic datasets. This approach could improve low-abundance gene detection and enable real-time monitoring of AMR trends.

5.3 Global Surveillance Networks

Expanding global surveillance networks for wastewater-based AMR monitoring could provide valuable insights into the spread and evolution of resistance genes. Collaborative initiatives could facilitate data sharing, methodological harmonization, and joint responses to emerging threats.

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6. Conclusion

Metagenomics holds significant promise for wastewater-based AMR surveillance, providing a comprehensive and nontargeted approach to monitoring resistance genes. However, challenges related to low-abundance gene detection, bioinformatics consistency, and environmental variability remain unresolved. Recent advances, such as the use of synthetic reference standards and machine learning-based data analysis, could address these limitations and improve the reliability and scalability of metagenomic surveillance. Combining qPCR and metagenomics, establishing standardized protocols, and expanding global surveillance networks will be critical steps toward effective AMR monitoring and management.

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