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Integrating Metagenomics in Environmental Monitoring: Co-Selection of Heavy Metal and Antimicrobial Resistance Genes in Contaminated Aquatic Systems

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Abstract: Heavy metal pollution and antimicrobial resistance genes (ARGs) are global challenges in aquatic ecosystems. They are commonly produced from agricultural waste, industrial effluents, and untreated sewage. Heavy metals, including cadmium, copper, and zinc, can exert pressure on microbial communities, leading to the co-selection of antimicrobial resistance genes (ARGs) and metal resistance genes (MRGs). This occurs through cross-resistance mechanisms, shared mobile genetic elements, and co-regulation under selective pressure. Metagenomics is known for its effectiveness and culture-independent nature, providing an advanced way to detect and profile ARGs and MRGs in environmental samples to facilitate the identification of functional genes and uncultured microorganisms. Findings from case studies from the Yamuna River (India) and Pear River (China) demonstrate strong relationships between concentrations of heavy metals and abundance of ARG/MRG, which supports the assumption that heavy metal contamination plays an integral role in sustaining antimicrobial resistance genes in aquatic systems. This paper also highlights metagenomics as a key tool for defining dynamics of co-selection, integrating biological indicators into monitoring water quality, and informing risk assessment. It establishes that standardized protocols, cross-sectoral frameworks, and long-term monitoring are vital for mitigating spread of antimicrobial resistance genes. Embedding genomics data with environmental parameters suggests that metagenomics support early interventions while strengthening environmental governance and minimizing public health risks for its environmental dissemination.

Keywords: *Metagenomics, Environmental Monitoring, Heavy metals, Antimicrobial Resistance Genes (ARGs), Aquatic Systems.*

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I. INTRODUCTION

Heavy metal pollution and antimicrobial resistance (AMR) constitute immense concerns in aquatic environments. Heavy metals can enhance antibiotic resistance by putting pressure on bacteria, resulting in the development of co-resistance methods (1). The concentrations of heavy metals and the abundance of antibiotic resistance genes in several aquatic ecosystems are strongly correlated (2). Similarly, the presence of microplastics exacerbates this issue as substrates for bacterial biofilms while accelerating the horizontal gene transfer of metal resistance genes and antimicrobial resistance genes (ARGs) (3).

Moreover, heavy metals in aquacultural and agricultural environments can drive the co-selection of antimicrobial resistance genes (ARGs) and metal resistance genes (MRGs) in the bacterial ecosystems. As heavy metals accumulate to concentrations in water and soil bodies with the potential to shape ARG soil profiles and trigger proliferation of antibiotic resistance (4), the co-occurrence of MRGs and ARGs can potentially enhance their co-selection in contaminated soils. This shows the urgent need to integrate public health and environmental strategies for mitigating pollutant-driven AMR in aquatic environments.

Although co-selecting ARGs and MRGs can pose a significant threat to public health in the aquatic environment (5), traditional monitoring only emphasises antibiotic toxicity but neglects the dynamics of the spread of AMR (6). On the other hand, metagenomics provides advantages in the effective investigation of AMRs, giving a more elaborate and robust understanding of the mechanisms of resistance and their context within the environment. Despite this, knowledge gaps remain in the roles of different metals, environmental conditions, and bacterial taxa in the co-selection processes (5). This paper aims to explore the role of metagenomics in co-selecting heavy MRGs and ARGs in polluted aquatic environments in order to enhance environmental monitoring while informing public health interventions.

II. HEAVY METAL POLLUTION AND ANTIMICROBIAL RESISTANCE IN AQUATIC SYSTEMS

Heavy metal pollution in water and soils is derived from anthropogenic and natural sources. According to (7), anthropogenic sources include industrial activities, excessive use of fertilizers and insecticides, and improper waste disposal, while natural sources are rock weathering and volcanic eruptions. It was observed by (8) that agricultural and mining practices, and industrial processes contribute to heavy metal pollution. Likewise, contaminants such as calcium, lead, zinc, and copper pose serious threats to plant growth, soil quality, human health, and the aquatic systems, especially as organic matter content and pH of the soil influence the metals' bioavailability and mobility (9).

Through co-selection mechanisms, heavy metal pollution in aquatic systems can lead to the emergence and spread of AMR, where antibiotic and MRGs are located on similar genetic elements, cross-resistance, such as shared defense mechanisms, and co-regulation where antibiotic resistance genes ARGs are activated by metal stress (10). While co-resistance is suggested as a major mechanism, the in-depth characterization is essential for confirming functional expression while connecting genes with unique bacterial hosts (5).

Taking the Pearl River in South China as a case study, metagenomic analyses show a strong relationship between ARGs and MRGs in sediments that are polluted with industrial effluents (11). High concentrations of copper, cadmium, and zinc metals co-occurred with some drug resistance genes, which suggests co-selection pressure. Also, network analysis showed the location of ARGs and MRGs on the same genetic elements, which indicates the likelihood of horizontal gene transfer between environmental bacteria.

In the case of Yamuna River, India, the site was impacted by untreated sewage and industrial discharge. By applying shotgun metagenomics, results show that there were high levels of MRGs in arsenic and mercury, as well as clinically relevant ARGs like sul1 and blaCTX-M (Parida et al., 2022). The study also demonstrated the positive correlation of concentrations of elevated metals and the abundance of ARG, which supports the hypothesis that heavy metal contamination maintains and spreads ARM in aquatic systems, especially due to lack of antibiotic inputs.

III. METAGENOMICS AS A TOOL FOR ENVIRONMENTAL MONITORING

Metagenomics has emerged as a tool for environmental monitoring based on its capacity for high-throughput and culture-independent analysis. This allows researchers to study uncultured microorganisms comprising the majority of biological diversity (13). Metagenomics has been successfully applied to several habitats, giving insights into ecosystem functioning and microbial community dynamics. With its high-throughput sequencing potential, metagenomics can simultaneously detect thousands of genetic markers and microbial taxa in just a single analysis, which gives a comprehensive overview of the ecosystem health and biodiversity (14).

In comparison with traditional culture-oriented methods, metagenomics is independent of culture, and this allows researchers to identify rare or uncultured microorganisms that may play critical roles in harbor resistance genes or ecological processes. This approach also allows for detecting functional genes such as ARGs and MRGs, which enable the examination of their diversity, abundance, and mobility in microbial populations (15). In addition, metagenomic datasets can be mined with the emergence of analytical tools, which enhances long-term monitoring value. The integration of sequence data with environmental parameters like PH, salinity, and pollutant concentrations is another important value of metagenomics, which supports early detection of emerging threats and holistic ecosystem assessments (16).

Shotgun metagenomic sequencing offers a comprehensive analysis of metabolic and community biodiversity pathways, where advanced sequencing technologies such as third-generation and next-generation sequencing have ensured rapid detection of microorganisms while improving taxonomic profiling (17). Novel bioactive genes and substances are screened by functional metagenomics, especially in identifying new ARGs from environmental samples (18). This is also useful in uncovering ARGs without existing sequencing knowledge, which contributes to the overall understanding of resistance mechanisms to identify new drug targets.

Moreover, bioinformatics approaches have transformed the detection and analysis of ARGs in environmental metagenomes. Pipelines and databases such as ARG-ANNOT, ResFinder, CARD, and SARG have been developed for this purpose (19). The tools, however, vary in their search algorithms, specificity, and data types (2). Quick annotation and classification of sequences like ARG from metagenomics data are achieved by ARGs-OAP, and while SARG is a relatively good database, the use of multiple tools can provide a comprehensive overview of the profiles in different environments. Bioinformatic tools are also used for global surveillance of pathogens and tracking AMRs, although it is vital to standardize the databases, phenotypic predictions, and pipelines to enhance and optimize the approaches (19).

IV. PUBLIC HEALTH AND ENVIRONMENTAL POLICY IMPLICATIONS

Metagenomics Environmental pathways are critical in the spread of AMR. In aquatic systems, this is due to antibiotic contamination, which occurs through different sources such as animal and human waste (20). The systems also play a role in the maintenance, mixing, and mobilization of resistance genes and AMR bacteria. Specifically, with environmental factors like pollutants and antibiotic residues contributing to the emergence and persistence of AMR mechanisms (21), the implications for public health and environmental policy include increased healthcare costs and treatment failures (20).

Besides, aquatic environments receiving industrial effluents, untreated sewage, and agricultural runoff are mixing grounds and reservoirs for ARGs and resistant pathogens. In their presence, the ARGs can spread to animal and human pathogens through horizontal gene transfer, which increases the risk of complex infections (22). Regarding environmental policy, persistent AMR in aquatic ecosystems highlights the need for integrated water quality management, which transcends chemical pollutant limits and includes biological risk indicators, including ARG abundance (23). Therefore, policies should be such that they promote stricter regulation of discharges, inclusion of AMR surveillance, and monitoring resistance markers in water bodies in national environmental monitoring schemes. A cross-sectoral approach that links environmental regulations with public health strategies, therefore, becomes essential as the failure to address the risks may result in widespread environmental dissemination of AMR, which undermines the efficacy of antibiotics while increasing healthcare costs and exacerbating inequities in global health.

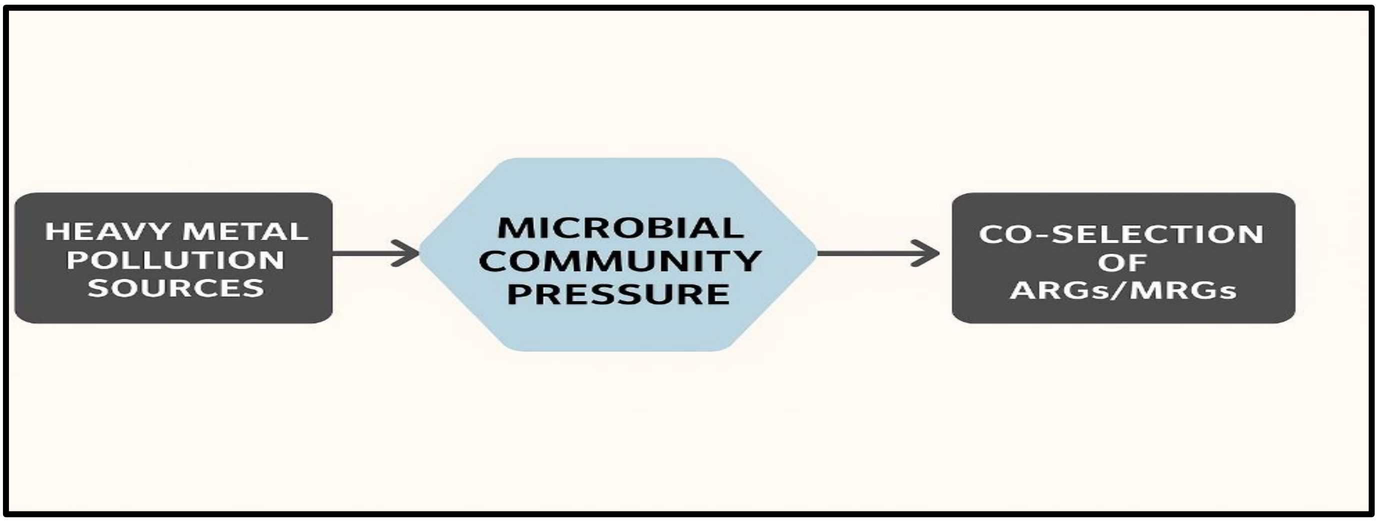

Fig 1 Illustration of Heavy Metal Pollution Leading to the Spread of Antimicrobial Resistance in Aquatic Systems.

Integrated monitoring frameworks can support policy recommendations for regulatory limits and improvement in wastewater treatment. First, the One Health, basin-wide design framework links animal, environmental, and human data across hospitals, wastewater plants, aquaculture, farms, and Major River reaches such as wet/dry and upstream downstream seasons (24). Also, multilayer analytics combine routine qPCR for sentinel markers, including sul1 and intl1, shotgun metagenomics, and targeted panels for priority MRGs/ARGs for function profiling and culture-based phenotypes for clinical pathogens. Risk-based triggers, such as tiered thresholds, escalate actions in the form of source tracing or treatment optimization in situations when ARG or MRG loads rise. Moreover, mobility + co-selection focus helps in tracking mobile genetic elements, metals, or antibiotics, and plasmidome fractions in order to quantify the pressure of co-selection (5). Lastly, open data and dashboards provide apparent real-time reporting to public health and water regulators with privacy safeguards.

Therefore, policy recommendations include embedding AMR metrics in permits for efficient discharge, publishing annual in-water reports on AMR, and providing funding for lab capacity, which aligns with AMR action plans across the nation (25). In addition, it is critical to provide stronger stormwater controls using green infrastructure, separation, and storage to prevent surges in AMR during overflows. Third, wastewater treatment can be upgraded or deploy advanced tertiary trains with activated carbon for micropollutants to ensure that disinfection achieves log-reductions (26).

V. CHALLENGES AND FUTURE DIRECTIONS

After Metagenomics has largely transformed environmental AMR monitoring, but some challenges persist in integrating it into routine monitoring. For instance, technical limitations such as sequencing depth, variability in sample processing, and bioinformatics pipelines complicate the comparison of studies. The absence of standardized protocols for detecting ARGs and MRGs, as well as their quantification, limits the reproducibility and applicability of results according to regulations (27). In addition, due to the complexity of data interpretation, metagenomics cannot sufficiently distinguish between dead cells, active expression, or extracellular DNA, which makes risk assessment uncertain (13).

Research gaps also persist in comprehending the long-term dynamics of MRG or ARG persistence in aquatic systems. Several studies are cross-sectional, which offer snapshots instead of transient trends that could show seasonal fluctuations, decay rates, or resilience. Mechanical insights into pathways of co-selecting the role of environmental stressors and mobile genetic elements in driving horizontal gene transfer are incomplete (5). More so, only a few studies link MRG or ARG loads to outcomes of clinical resistance, which limits the evidence for policy interventions.

Additionally, emerging technologies offer promising solutions. Resistance genes can be linked directly to host mobile and taxa elements, which can help to improve source attribution. Artificial intelligence (AI) analysis and machine learning (ML) can integrate chemical, multi-omics, and hydrological datasets for predicting AMR hotspots as well as risks pertaining to co-selection (28). Portable sequencing platforms, including nanopore devices, may facilitate on-site monitoring to reduce sampling-decision-making lag times.

Therefore, future work should focus on global standardization efforts, mechanical experiments, and longitudinal studies spanning many years under realistic conditions. Integrating advanced sequencing with predictive analytics could also help in transforming AMR monitoring from a reactive to a preventive framework with the aim of fostering earlier interventions and robust environmental and public health protection.

VI. CONCLUSION

Heavy metal contamination in aquatic systems helps in sustaining and spreading antimicrobial resistance (AMR) through the co-selection of antimicrobial resistance genes (ARGs) and metal resistance genes (MRGs). Evidence from studies on metagenomics highlights strong co-occurrence of gene patterns, which are attributed to urban, industrial, and agricultural pollution sources. Metagenomics proves indispensable in monitoring the resistance determinants, showing their diversity, abundance, mobility, and relationship with mobile genetic elements in cultured and uncultured microorganisms. In comparison with traditional culture-based approaches, metagenomics provides a high-resolution and comprehensive perspective of functional genes and microbial communities, which enhances the detection of the co-selection processes.

Despite the benefits obtainable from the integration of metagenomics in environmental monitoring, gaps persist in the standardization of analytical protocols, the establishment of clear policies, and the interpretation of complex datasets. Integrating advanced sequencing with single-cell genomics, artificial intelligence, and portable platforms provides promising avenues for effective monitoring and predictive modelling. Antimicrobial resistance (AMR) can be addressed in aquatic systems, which demands interdisciplinary collaboration between environmental scientists, microbiologists, policymakers, and public health experts. Regulatory measures should include the incorporation of ARGs and MRGs surveillance into water quality metrics, enforcing stricter discharge controls, and strengthening wastewater treatment. Finally, embedding metagenomics into monitoring frameworks can foster effective AMR management by safeguarding the integrity of the aquatic ecosystems and global public health.

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