Review

Critical perspectives on advancing antibiotic resistant gene (ARG) detection technologies in aquatic ecosystems

Zainab N. Nassereddine , Somie D. Opara , Oliver A. Coutinho , Florent Qyteti , Reeghan Book , Matthew P. Heinicke , Jacob Napieralski , Sonia M. Tiquia-Arashiro *

Department of Natural Sciences, University of Michigan-Dearborn, 4901 Evergreen Road, Dearborn, MI 48124, USA

HIGHLIGHTS

- Unveils new molecular methods advancing ARG monitoring in aquatic systems
- Discusses integration of bioinformatics into ARG detection methodologies
- Highlights environmental biosensors' role in real-time ARG surveillance
- Critiques current limitations and future needs for ARG monitoring techniques
- Advocates interdisciplinary approaches to improve ARG tracking in ecosystems

GRAPHICAL ABSTRACT
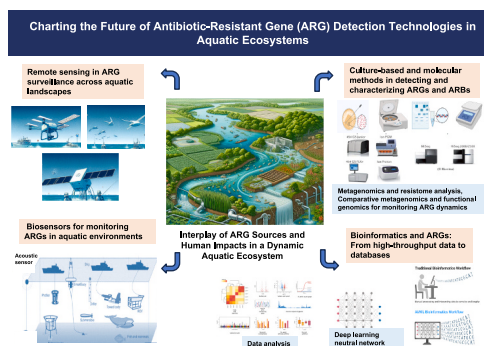
ARTICLE INFO

ABSTRACT

The spread of antibiotic resistance genes (ARGs) in aquatic ecosystems poses a serious risk to environmental and public health, making advanced detection and monitoring methods essential. This review provides a fresh perspective and a critical evaluation of recent advances in detecting and monitoring ARGs in aquatic environments. It highlights the latest innovations in molecular, bioinformatic, and environmental techniques. While traditional methods like culture-based assays and polymerase chain reaction (PCR) remain important, they are increasingly being supplemented by high-throughput sequencing technologies applied to metagenomics. These technologies offer comprehensive insights into the diversity and distribution of ARGs in aquatic environments. The integration of bioinformatic tools and databases has improved the accuracy and efficiency of ARG detection, enabling the analysis of complex datasets and tracking the evolution of ARGs in aquatic settings. Additionally, new environmental monitoring methods, including novel biosensors, geographic information systems (GIS) applications, and remote sensing technologies, have emerged as powerful tools for real-time ARG surveillance in water systems. This review critically examines the challenges of standardizing these methodologies and emphasizes the need for interdisciplinary approaches to enhance ARG monitoring across different aquatic ecosystems. By assessing the strengths and limitations of various methods, this review aims to guide future research and the development of more effective strategies for managing antibiotic resistance in aquatic environments.

1. Introduction

The presence of resistance genes (ARGs) in aquatic ecosystems is a critical environmental and public health issue. Aquatic environments such as rivers, lakes, and oceans serve as reservoirs for ARGs, facilitating their spread across diverse microbial communities and potentially into human populations (Shin et al., 2023; Wang et al., 2024). The presence of ARGs in these ecosystems is often linked to anthropogenic activities such as agricultural runoff, wastewater discharge, and pharmaceutical contamination, which introduce antibiotics and antibiotic-resistant bacteria (ARBs) into water bodies (Givens et al., 2023). This necessitates the need for robust methodologies to detect and monitor ARGs in aquatic settings.

Over the past decade, significant progress has been made in this area, driven by innovations in molecular biology, bioinformatics, and environmental science. Traditional methods such as culture-based assays and polymerase chain reaction (PCR) continue to play a crucial role in ARG detection (Ahmad et al., 2024). These techniques are increasingly being complemented and, in some cases, replaced by high-throughput sequencing technologies (Zhao et al., 2023). Metagenomics and whole-genome sequencing offer comprehensive insights into the diversity and distribution of ARGs, enabling a more detailed understanding of their presence and dynamics in water bodies. However, these innovations face challenges in sample processing complexity and the need for robust bioinformatics to handle large datasets. This review synthesizes recent advances in detecting ARGs in aquatic systems, examines current methodological challenges, and discusses future strategies to enhance our ability to track and combat this growing problem. The integration of sophisticated bioinformatic tools and databases has further revolutionized ARG detection. These technologies enhance the accuracy and efficiency of analyses, allowing researchers to manage and interpret complex datasets and track the evolution of ARGs over time (Abdi et al., 2024). Additionally, novel environmental monitoring approaches, such as biosensors and remote sensing technologies, have emerged as powerful tools for real-time ARG surveillance. These innovations provide timely and actionable data, which are crucial for managing the spread of antibiotic resistance (Zhang et al., 2024). Despite these advancements, several challenges remain.

Highlighting recent progress, this review explores new biosensor technologies that enable sensitive, real-time detection of ARGs in diverse aquatic environments. It stresses the importance of global standards for data collection and interpretation to accurately assess antibiotic resistance trends and their impact on ecology and public health. Additionally, the review underscores how cloud computing and AI are improving predictive models for tracking ARG dynamics, offering potential for proactive resistance management. It also examines the importance of Geographic Information Systems (GIS) and remote sensing technologies in monitoring ARGs, while acknowledging challenges associated with these methods.

This review offers a fresh perspective and critical evaluation of the latest advancements in ARG detection and monitoring in aquatic ecosystems. It highlights the strengths and limitations of current techniques and discusses future research directions, emphasizing the need for standardized approaches and interdisciplinary collaboration. Ultimately, the review aims to inform future research directions and the development of more effective strategies for managing antibiotic resistance.

2. Overview and significance of ARGs in aquatic ecosystems

The discovery of penicillin by Alexander Fleming in 1928 marked the beginning of the antibiotic era, revolutionizing health care and agriculture. However, overuse and misuse of antibiotics quickly led to the emergence of resistant bacteria, first detected in aquatic environments and drinking water by the 1950s (Bell et al., 1980; Armstrong et al., 1981). Industrial and agricultural activities, including wastewater

discharge, are known to be significant contributors to the spread of ARGs in water bodies (Catalfano et al., 2025; Patel et al., 2025). Human influences such as agricultural runoff and sewage introduce antibiotics and antibiotic-resistant bacteria (ARBs) into freshwater ecosystems, with hospital sewage particularly rich in antibiotics posing a notable threat (Lien et al., 2017). Despite efforts by wastewater treatment plants (WWTPs) to remove pollutants, they often fail to eliminate antibiotics, resulting in the release of ARBs and ARGs in effluents (Rowe et al., 2017). Rivers, affected by runoff and WWTP discharges, serve as conduits for both ARBs and ARGs (Catalfano et al., 2025; Chamlee et al., 2025). Additionally, mobile genetic elements (MGEs), like plasmids and integrons, facilitate the spread of ARGs through horizontal gene transfer (HGT) in aquatic environments (Catalfano et al., 2025). Groundwater, a critical source of drinking water, is also affected by ARG contamination due to infiltration from surface water and leaching from agricultural and industrial sites. Studies have shown that groundwater near intensive farming operations and wastewater treatment facilities exhibit elevated levels of ARGs (Pruden et al., 2006, 2013; Tiquia, 2010; Muziasari et al., 2016; Ferraro et al., 2024). Oceans, too, are increasingly recognized as reservoirs for ARGs (Chen et al., 2013; Louca et al., 2016; McKindles and Tiquia-Arashiro, 2012; Tiquia-Arashiro, 2012; Xu et al., 2023). Coastal waters receive significant inputs from river discharges, urban runoff, and treated effluents, which introduce ARBs and ARGs into marine environments (Tiquia-Arashiro et al., 2025). The widespread distribution of ARGs in marine settings poses risks for marine life and human health, especially considering the potential for ARGs to be transferred among marine microbial communities through HGT (Tiquia-Arashiro, 2012; Xu et al., 2023).

ARGs in aquatic ecosystems threaten biodiversity, ecosystem functions, and public health by contributing to hard-to-treat infections transmitted through contaminated water, recreational activities, and food consumption (Nnadozie and Odume, 2019). Case studies in various regions highlight the link between high ARG levels in water and resistant infections in local populations, underscoring the global implications of this issue (Muziasari et al., 2016). For example, in Finland, municipal and industrial wastewater discharge has been shown to introduce high concentrations of ARGs into waterways, especially from hospitals and pharmaceutical plants (Muziasari et al., 2016). In China, agricultural practices contribute significantly, as demonstrated by studies showing that livestock antibiotics promote ARB development, and manure runoff contaminates soil and water (Zhu et al., 2013; Nguyen et al., 2013). Additionally, urban stormwater runoff and sewage overflows during heavy rains have been identified as key factors exacerbating the spread of ARGs in densely populated cities in the United States (Liu et al., 2021). Moreover, standard wastewater treatments often inadequately remove ARGs, leading to their environmental release as observed in Portugal (Manaia et al., 2018).

Metals and xenobiotics, such as biocides and heavy metals, exacerbate antibiotic resistance in microbial communities through co-selection mechanisms (Bowman et al., 2018; Pagnucco et al., 2023; Opara et al., 2025). These compounds can induce stress responses that upregulate genetic elements (Kassem et al., 2023; Pagnucco et al., 2023) facilitating HGT of ARGs, thereby enhancing their spread and persistence in the environment (Buffet-Bataillon et al., 2016). Environmental factors like temperature, pH, pollutants, and climate shifts further influence ARG dynamics, creating environments conducive to ARB proliferation (Nnadozie and Odume, 2019; Magnano San Lio et al., 2023; Sanchez et al., 2025). Addressing the complex interplay between metals, xenobiotics, and microbial stress responses is crucial for effectively managing antibiotic resistance. Moreover, integrated strategies such as advanced wastewater treatment, reduced agricultural runoff, and sustainable practices are essential to mitigate the spread of ARGs in aquatic environments (Pruden et al., 2013; Oest et al., 2018; Tiquia-Arashiro, 2018; Patel et al., 2019; Alam et al., 2021).

Table 1

Research goals, challenges, and limitations of PCR and qPCR techniques for ARG and ARB detection in the literature.

PCR/qPCR method	Research goal	Challenges/limitations
Conventional PCR	Identify management strategies for controlling the spread of antibiotic-resistant genes (ARGs) and bacteria (ARBs) via environmental pathways, aiming to prolong the effectiveness of antibiotics.	Difficulty in quantifying long-term benefits of specific interventions. The link between mitigation efforts and clinical treatment outcomes is uncertain.
Multiplex PCR	Detect multiple ARGs simultaneously in bacterial species to assess resistance patterns in environmental settings, such as rivers or wastewater.	Limited amplification capacity for a large number of fragments. Cross-reactivity and primer specificity issues may hinder accurate species identification in mixed cultures.
Digital PCR (dPCR)	Conduct high-resolution quantification of ARGs in environmental samples, providing a tool for robust surveillance and tracking resistance in urban waterways and wastewater treatment plants (WWTPs).	Variability in results due to methodological differences, such as translating absolute dPCR data to relative metagenomic abundance. High sensitivity but challenges in establishing direct gene-host associations.
Droplet Digital PCR (ddPCR)	Compare various PCR techniques, focusing on efficiency, sensitivity, and ARG analysis capabilities, in a variety of environmental matrices.	Challenges with high GC content in target genes, potential bias in amplification, and issues with Poisson distribution analysis for certain DNA concentrations.
Loop-Mediated Isothermal Amplification (LAMP)	Develop and validate rapid, isothermal methods for detecting ARGs, offering an alternative to PCR that can be used in field settings.	Dependence on primer matching for known target sequences, leading to potential false negatives if ARGs have mutated. Variations in assay sensitivity and specificity for novel or emerging ARG variants.
Conventional qPCR	Quantify specific ARGs in environmental matrices like wastewater, to assess trends in resistance and support regulatory decision-making.	Protocol variations across studies lead to inconsistencies in detection limits and quantification. Environmental inhibitors often interfere with assay performance.
High-throughput qPCR (HT-qPCR)	Conduct large-scale screening of ARGs across diverse environmental samples, enhancing our understanding of pollution sources and the dynamics of ARG spread.	Variability in DNA extraction methods can impact results, particularly for low-abundance genes. Lack of standardized protocols complicates cross-study comparisons.
Multiplex qPCR	Enable rapid, simultaneous detection of multiple ARGs and mobile gene elements (MGEs) to track contamination in complex environments like rivers or wastewater.	Amplification issues such as non-specific signals or failure to amplify certain target genes, leading to false negatives. Difficulty in accurately quantifying complex ARG profiles in environmental samples.

3. Molecular methods in detecting and characterizing ARGs and ARBs

Historically, culture-based methods have been key in identifying antimicrobial resistance (AMR), starting with penicillin resistance and beta-lactamase enzymes in *Staphylococcus aureus* (Abraham and Chain, 1988). The Kirby-Bauer disk diffusion technique, standardized in the 1960s, enabled detailed AMR analysis (Bauer et al., 1966). Molecular methods later revealed ARGs on mobile elements like plasmids (Levy et al., 1976). Despite genomic advances, culture methods remain essential for validating resistance and understanding bacterial biology (Bonnet et al., 2020). Recent innovations include Lab-on-a-Chip devices for rapid antibiotic tests (Mark et al., 2010) and automated systems like BD Phoenix™ with digital imaging for fast, standardized results (Idelevich and Becker, 2019). These miniaturized devices integrate various laboratory functions on a single chip, allowing for the rapid detection of antibiotic resistance genes (ARGs) in a small sample. By using microfluidics, they can perform multiple tests simultaneously, providing quick and accurate identification of resistant bacteria. This technology is particularly useful for on-site testing, enabling immediate decision-making in clinical and environmental settings (Tiquia-Arashiro and Pant, 2020). Automated systems like BD Phoenix™ also represent significant advancements. The BD Phoenix™ system utilizes digital imaging and advanced algorithms to analyze microbial growth and determine antibiotic susceptibility. By rapidly identifying bacterial strains and their resistance profiles, it provides standardized and reliable results. This system helps in the early detection of ARGs, facilitating timely and appropriate treatment decisions, thereby combating the spread of antibiotic-resistant infections. Both innovations enhance our ability to detect and monitor antibiotic resistance, playing a crucial role in addressing the growing public health threat posed by ARGs. However, culture methods fail to detect non-cultivable bacteria, missing many relevant ARGs (Sommer et al., 2010). This affects treatments and control practices due to hidden ARGs in human microflora and biofilms (Perry et al., 2004). Strategies like new media formulations or co-cultures can enhance bacterial cultivation (Demin et al., 2024; Song et al., 2024).

To address these limitations, molecular methods such as PCR-based techniques, metagenomics, metatranscriptomics, and single-cell genomics have been used to detect ARGs in non-cultivable organisms. High-throughput sequencing enables comprehensive screening of microbial communities for ARGs, biogeographical studies, and tracking resistance evolution. Functional metagenomics identifies novel ARGs by cloning environmental DNA into expression vectors followed by functional screening in a culturable host.

3.1. PCR and quantitative PCR (qPCR) for targeted ARG detection

PCR and qPCR techniques have been successfully employed to detect specific antibiotic resistance genes (ARGs) in various aquatic environments (Galhano et al., 2021). Pruden et al. (2006) used conventional PCR to identify tetracycline resistance genes like *tet*O, *tet*M, and *tet*Q in sediment and groundwater contaminated by swine manure. Munir et al. (2011) quantified *erm*B, *sul*I, and *sul*II ARGs in wastewater and surface water using qPCR, assessing their fate in wastewater treatment. Wang et al. (2016) utilized reverse transcription quantitative polymerase chain reaction (RT-qPCR) to measure ARG expression levels in river water, revealing gene activity. Wang et al. (2018) used droplet digital PCR (ddPCR) to quantify *str*A (streptomycin resistance), *mec*A (methicillin and other beta-lactam antibiotics resistance), and *van*A (vancomycin resistance) at 13 monitoring sites along the Weihe River in China, demonstrating accurate ARG measurement.

Recent advancements in PCR-based methodologies have significantly improved the detection and quantification of antibiotic resistance genes (ARGs) in aquatic environments (Table 1). The precision, sensitivity, and throughput of these methods now allow for the identification of ARGs across a broad range of samples, even at very low concentrations. Fig. 1 illustrates the evolution of PCR technologies from traditional qPCR to advanced methods such as digital PCR (dPCR), multiplex PCR, Loop-Mediated Isothermal Amplification (LAMP), and high-throughput quantitative PCR (HT-qPCR) (Mu et al., 2016; Park et al., 2021; Kasuga et al., 2022; Carrasco-Acosta and Garcia-Jimenez, 2024; Srathongneam et al., 2024). Digital PCR (dPCR) provides absolute quantification of ARGs without standard curves, achieving unmatched precision (Keenum et al., 2022; Maestre-Carballa et al., 2024). Multiplex PCR allows for simultaneous amplification of multiple ARGs in one reaction, improving screening efficiency (Stedtfeld et al., 2018). Zhang
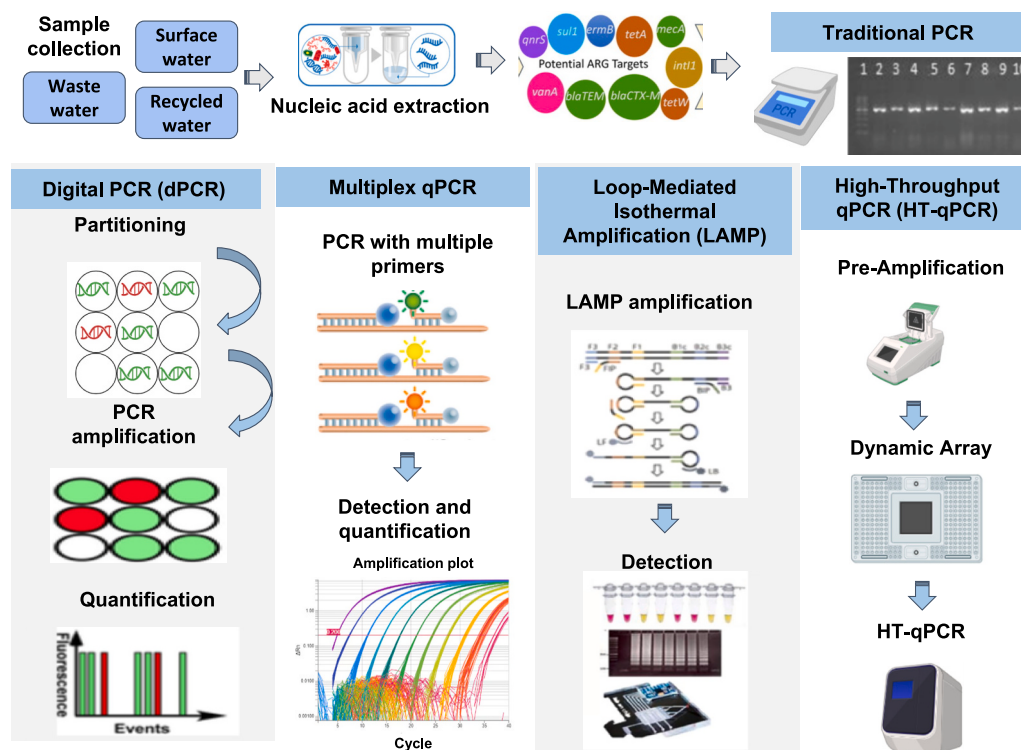
Fig. 1. Recent advancements in PCR-based methodologies for detecting antibiotic resistance genes (ARGs) in aquatic environments. The figure illustrates the evolution of PCR technologies from traditional qPCR to advanced methods such as digital PCR (dPCR), multiplex PCR, Loop-Mediated Isothermal Amplification (LAMP), and high-throughput quantitative PCR (HT-qPCR). Case studies include Keenum et al. (2022) on dPCR's precision in diverse water matrices, Stedtfeld et al. (2018) showcasing HT-qPCR arrays for robust ARG detection in rivers, and Luo et al. (2010) demonstrating LAMP's field-applicable detection of sulfonamide resistance genes in aquaculture settings.

et al. (2009) developed a multiplex PCR for detecting multiple ARGs (*erm*B, *erm*F, *erm*Q, *msr*A, *mef*A, and *van*A) in the Yangtze Estuary. LAMP innovations offer quick and accurate on-site ARG detection, as shown by Luo et al. (2010) and Hassan et al. (2023), targeting sulfonamide and β-lactam resistance genes, respectively. HT-qPCR platforms, enhanced by microfluidic technology, enable rapid amplification and quantification of numerous ARGs across multiple samples, as demonstrated by Stedtfeld et al. (2018). These advancements are crucial for effective ARG monitoring in aquatic environments. They provide essential insights into ARG spread and persistence, guiding water treatment practices and public health responses to antibiotic resistance.

Despite advancements, PCR-based methods for detecting ARGs in aquatic environments face challenges in consistency and comparability (Table 1). Environmental heterogeneity can skew detection, while variations in DNA extraction may miss low-abundance genes (Daw Elbait et al., 2024). The lack of standardized PCR protocols, including multiplex PCR and HT-qPCR, complicates cross-study comparisons (Daw Elbait et al., 2024). Different PCR assays and non-PCR methods like LAMP add to data interpretation complexity, requiring a unified analytical framework. Quality control in high-throughput settings like HT-qPCR is essential but resource-intensive. To improve detection, standardization is crucial. Researchers should adopt detailed protocols that account for environmental variation and use standardized DNA extraction techniques to capture low-abundance ARGs (Daw Elbait et al., 2024). Consensus on PCR protocols, primers, and amplification conditions will enhance consistency (Daw Elbait et al., 2024). Comprehensive ARG annotation databases and accessible bioinformatics tools are essential. Quality control benchmarks ensure high data quality, leading to more reliable and comparable findings (Caporaso et al., 2012).

3.2. Metagenomics and resistome analysis

Metagenomics is crucial for studying the resistome—all antibiotic resistance genes in a microbial community—by enabling detection and quantification of resistance genes without needing specific primers for PCR amplification (Ferrario et al., 2017). Fig. 2 illustrates the workflow for metagenomic-based monitoring of ARGs in aquatic environments. Studies have shown metagenomics effectively characterizes the resistome in diverse environments. In São Paulo, Brazil, metagenomic profiling of soil resistomes revealed significant differences in microbial communities and ARGs due to agricultural activities (Ordine et al., 2023). Metagenomics of human gut microbiomes from 26 case-control studies found higher ARG abundances in patients with diseases treated by antibiotics, like cystic fibrosis and diarrhea, compared to controls (Fredriksen et al., 2023). In Moscow, metagenomic analysis of wastewater treatment plants identified numerous ARGs in untreated wastewater, with treatment processes significantly reducing ARG abundance, highlighting the role of treatment technologies in managing antibiotic resistance (Begmatov et al., 2024).

Advancements in metagenomics have significantly enhanced our understanding of the pervasiveness and diversity of ARGs in aquatic ecosystems (Table 2). These advancements enable the discovery of new ARGs and illustrate the impact of human activity on resistance spread (Ng et al., 2017; Chen et al., 2019a, 2019b). Kristiansson et al. (2011) used metagenomics to uncover numerous ARGs in river sediments affected by wastewater discharge, highlighting its capacity to reveal novel ARGs. Pehrsson et al. (2016) identified biofilms in drinking water systems as ARG hotspots. Xiong et al. (2015) demonstrated the risks posed by aquaculture in propagating ARGs, while Bengtsson-Palme et al. (2016) showed how sewage treatment processes influence the resistome, necessitating improved treatment methods. Advancements in sequencing technologies and bioinformatics offer detailed ARG
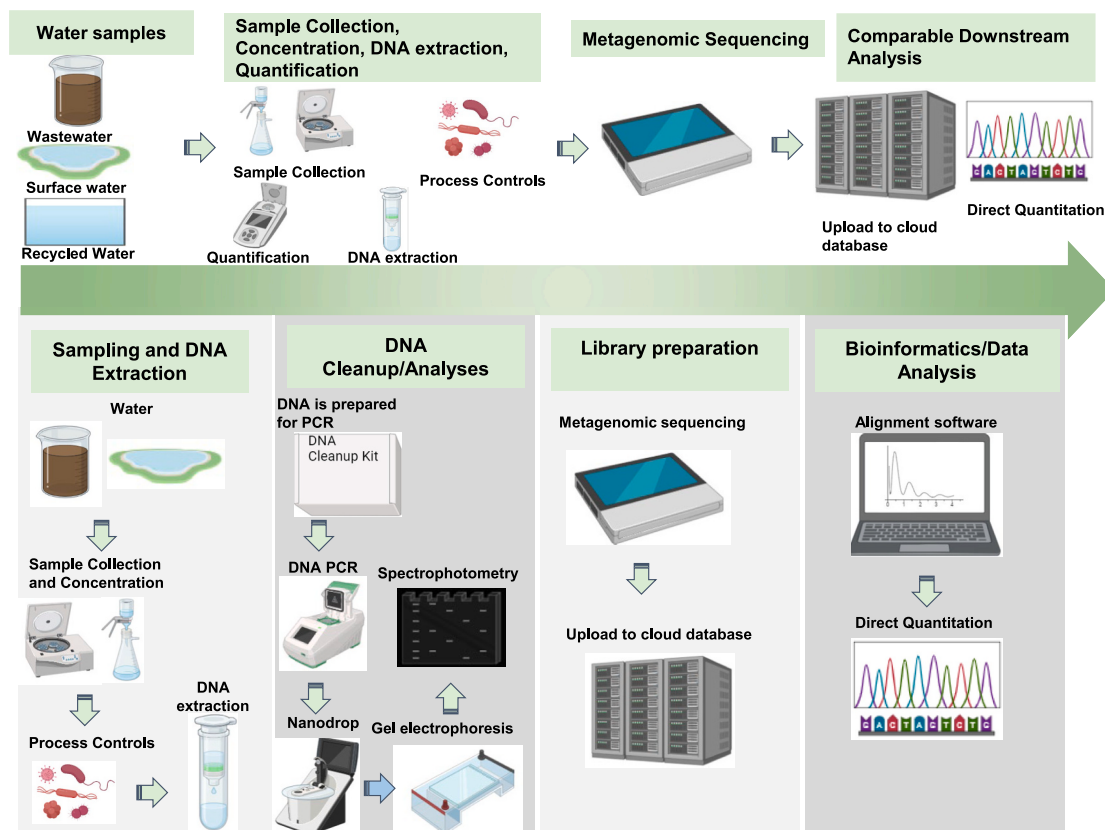
Fig. 2. Overview of workflow for metagenomic-based monitoring of ARGs in aquatic environments that are highlighted in this review. Adapted from Davis et al. (2023).

characterization, enabling more accurate mapping within microbial communities. Chen et al. (2013) profiled ARGs in coastal waters, essential for assessing threats to ecosystem and human health. These developments enhance our ability to monitor, characterize, and mitigate ARG spread in aquatic ecosystems. Challenges in these metagenomic studies include managing large, complex datasets, requiring advanced bioinformatics and careful database selection for accurate gene detection (Ferrario et al., 2017; Rodrigues et al., 2018). Limited genome coverage in diverse communities and difficulty separating microbial DNA from host contamination complicate analysis (Galhano et al., 2021). Enhanced bioinformatics tools, better databases, and methods to improve genome coverage and DNA separation are needed to address these issues.

3.3. Functional genomics

Functional genomics plays a crucial role in understanding ARGs within aquatic ecosystems by studying gene functions and interactions on a genomic scale. For example, Amos et al. (2014) showed that functional genomics could elucidate how microbial communities in rivers adapt and develop resistance. A study by Parsley et al. (2010) employed functional genomics to screen for ARGs in activated sludge in wastewater treatment plants (WWTPs). They identified novel genes conferring resistance to a variety of antibiotics, broadening our understanding of resistance within WWTP microbial communities. Kristiansson et al. (2011) used functional metagenomics to investigate the presence of ARGs in the sediment of rivers exposed to industrial pharmaceutical waste. This approach revealed a variety of ARGs and provided a snapshot of the potential risk posed by pharmaceutical contamination to the spread of antibiotic resistance. A functional genomic analysis by Eduardo-Correia et al. (2020) studied ARGs in multi-contaminated Tinto River, revealing a reservoir of multi-drug

resistance genes in the bacterioplankton community, which are potentially involved in horizontal gene transfer. A study by Marano et al. (2020) conducted functional metagenomic screening on ARGs in urban wastewater treatment plants. The research highlighted the effluent as a hotspot for resistance determinants, including novel genes not previously associated with clinical pathogens.

Recent advancements in functional genomics have significantly improved the study of ARB and ARGs in aquatic environments (Table 3). CRISPR-Cas systems enable precise editing and knockdowns of specific genes, providing insights into ARB function and ARG expression mechanisms (Quan et al., 2019; Gupta et al., 2024; Long et al., 2024; Mao et al., 2024; Shin et al., 2024). CRISPR-Cas systems are considered as barriers to HGT in bacteria (Zheng et al., 2020). Crispr-Cas system can be used to re-sensitize drug-resistant bacteria to antibiotics by specifically eliminating the plasmids carrying antibiotic resistance genes (Gholizadeh et al., 2020). The CRISPR-Cas system has potential applications in preventing and controlling the spread of bacterial resistance caused by drug resistance genes (Tao et al., 2022). Its direct application involves designing genomic gRNAs to target drug-resistant genes or resistant bacteria, guiding the CRISPR-Cas system to cleave these sequences. Fig. 3 illustrates the working mechanism of the CRISPR-Cas system and its application in addressing antibiotic resistance This process can restore antibiotic sensitivity or kill the bacteria (Pickar-Oliver and Gersbach, 2019). A major challenge is the efficient delivery of the CRISPR-Cas system into microorganisms to combat bacterial drug resistance (Lino et al., 2018).

Another advancement in functional genomics is that single-cell sequencing allows for the examination of ARG presence and variability at the single-cell level, overcoming the limitations of bulk sequencing (Blainey, 2013). Integrating metagenomics with metatranscriptomics, metaproteomics, and metabolomics offers a comprehensive understanding of ARB and ARGs, linking genomic potential to active

Table 2

Research goals, challenges, and limitations of metagenomic techniques used in detection and characterization of ARGs and ARBs in the literature.

Research goal	Challenges and limitations
To identify and reconstruct ARGs directly from metagenomic data.	Metagenomic approaches like fARGene rely on sequence homology with known ARGs, potentially missing novel or divergent resistance genes. Additionally, inconsistent sample types and sequencing depth may affect data quality and completeness.
To recover genome sequences of drug-resistant bacteria from metagenomic samples.	Variability in sample types (e.g., fecal samples, blood, cerebrospinal fluid) can affect microbial complexity and consistency of results, making data quality a persistent challenge.
To detect ARGs and resistance-conferring SNPs in bacterial genomes and metagenomic samples using short-read Illumina sequencing.	Sufficient sequencing depth is necessary to detect low-abundance ARGs. Insufficient depth can lead to missing clinically significant ARGs, limiting the detection of less abundant resistance genes.
To study the prevalence and diversity of ARGs in eutrophic urban lake sediments.	While metagenomic sequencing can identify ARGs not detected by qPCR, sensitivity for low-abundance ARGs is limited, leading to a potential underrepresentation of clinically relevant genes.
To use machine learning (random forests) to predict associations between ARGs and bacterial taxa in wastewater treatment plant samples.	Complex, nonlinear relationships between ARGs and bacterial taxa complicate analysis. Advanced tools are needed to model these associations, making interpretation challenging.
To explore the impact of human activities on ARG spread through comparative analysis.	Metagenomic data provides genetic profiles but cannot confirm functional activity of detected ARGs. Experimental validation is required to confirm their relevance in resistance processes.
To compare ARGs in hospital wastewater, municipal wastewater, and urban surface waters.	Database reliance for ARG identification may overlook novel or underrepresented resistance genes. This bias affects accuracy and comparability, leading to potential underestimation of novel resistance profiles.
To examine changes in ARG abundance and diversity before and after a flood event using high-throughput sequencing.	Environmental sample diversity and complex physicochemical factors complicate the interpretation of ARG data, making it difficult to directly attribute observed changes to the flood event.
To compare ARGs in river sediments with those in pristine environments (e.g., Antarctic soils, deep-sea sediments) to assess the impact of human activity.	Insufficient exploration of ARG interactions with mobile genetic elements and environmental factors limits the understanding of the mechanisms driving ARG dissemination, particularly in complex natural environments.

metabolic pathways and gene expression profiles (Liu et al., 2019). Advancements in sequencing technologies, such as nanopore and PacBio sequencing, provide longer reads and real-time data analysis, useful for reconstructing full ARGs and their vectors within microbial genomes (van Dijk et al., 2018). Artificial intelligence and machine learning help manage and interpret complex datasets, predicting ARG function from sequence data, identifying novel resistance determinants, and modeling the spread and evolution of resistance in aquatic environments (Camacho et al., 2018; Sun et al., 2021). These advanced techniques are transforming our ability to study ARB and ARGs, offering deeper insights into antibiotic resistance mechanisms and spread.

Using functional genomics to study aquatic environments offers valuable insights but also presents challenges. The immense diversity and dynamic nature of aquatic microbial communities require extensive sampling and deep sequencing for thorough coverage (Sunagawa et al., 2015). Degraded DNA and RNA in these samples can reduce sequencing accuracy, especially for transient gene expressions (Huggett et al., 2015). Analyzing large-scale datasets demands significant bioinformatics resources and expertise, creating accessibility issues (Quince et al., 2017). The accuracy of functional annotation relies on high-quality reference databases, which are often incomplete for non-model organisms, leading to unidentified genes and underestimated diversity (Eisen, 2015). Additionally, the resource-intensive nature of sequencing and data processing limits the depth and frequency of sampling (Goodwin et al., 2016).

Table 3

Research goals, challenges, and limitations of functional genomic techniques used in detection and characterization of ARGs and ARBs in the literature.

Method	Research Goal	Challenges and limitations
Functional metagenomics	To assess the impact of wastewater treatment plant effluent on the diversity and abundance of ARGs in a riverine environment.	While functional metagenomics can identify a broad range of resistance mechanisms, ecological and evolutionary implications of these findings require more in-depth study. Combining functional metagenomics with other omics approaches (e.g., transcriptomics, proteomics) could provide more comprehensive insights into the expression and regulation of ARGs in environmental contexts.
Proteomics and metaproteomics	To investigate the factors driving the accumulation and transmission of ARGs in biofiltration systems, particularly those involving biochar.	The diversity of microbial genera and their varied metabolic behaviors can make interpreting proteomic data challenging. The same genus may exhibit different ARG carriage patterns under different environmental conditions, complicating the identification of universal drivers of resistance.
CRISPR-Cas systems for functional genomics	To develop and validate FLASH, a CRISPR/Cas9-based method for enriching and detecting low-abundance pathogen sequences, particularly AMR genes, in clinical samples.	The performance of FLASH-NGS is hindered by the complexity of clinical samples, where the microbial component is often present at low abundance compared to the host genomic background. This leads to reduced on-target read proportions, affecting the sensitivity and accuracy of pathogen detection, especially in mixed clinical samples.
	To develop a sensitive, specific, and rapid method for detecting AMR bacteria with blaNDM genes using a CRISPR/Cas12a-based fluorescence assay.	The assay's performance depends heavily on precise optimization of several components, including the Cas12a, gRNA, and ssDNA FQ-reporter ratios. Variability in these parameters can affect the consistency and reliability of the assay, limiting its broad application without extensive standardization and optimization.
	To create a portable biosensor combining CRISPR/Cas12a and LAMP for on-site detection of ARGs in wastewater.	The method requires extensive validation across a wide range of wastewater samples from various sources (e.g., industrial, agricultural runoff) to ensure robustness and reliability. Variability in the composition of wastewater from different environments may affect the accuracy of the biosensor, necessitating diverse sample testing and environmental condition adaptation.
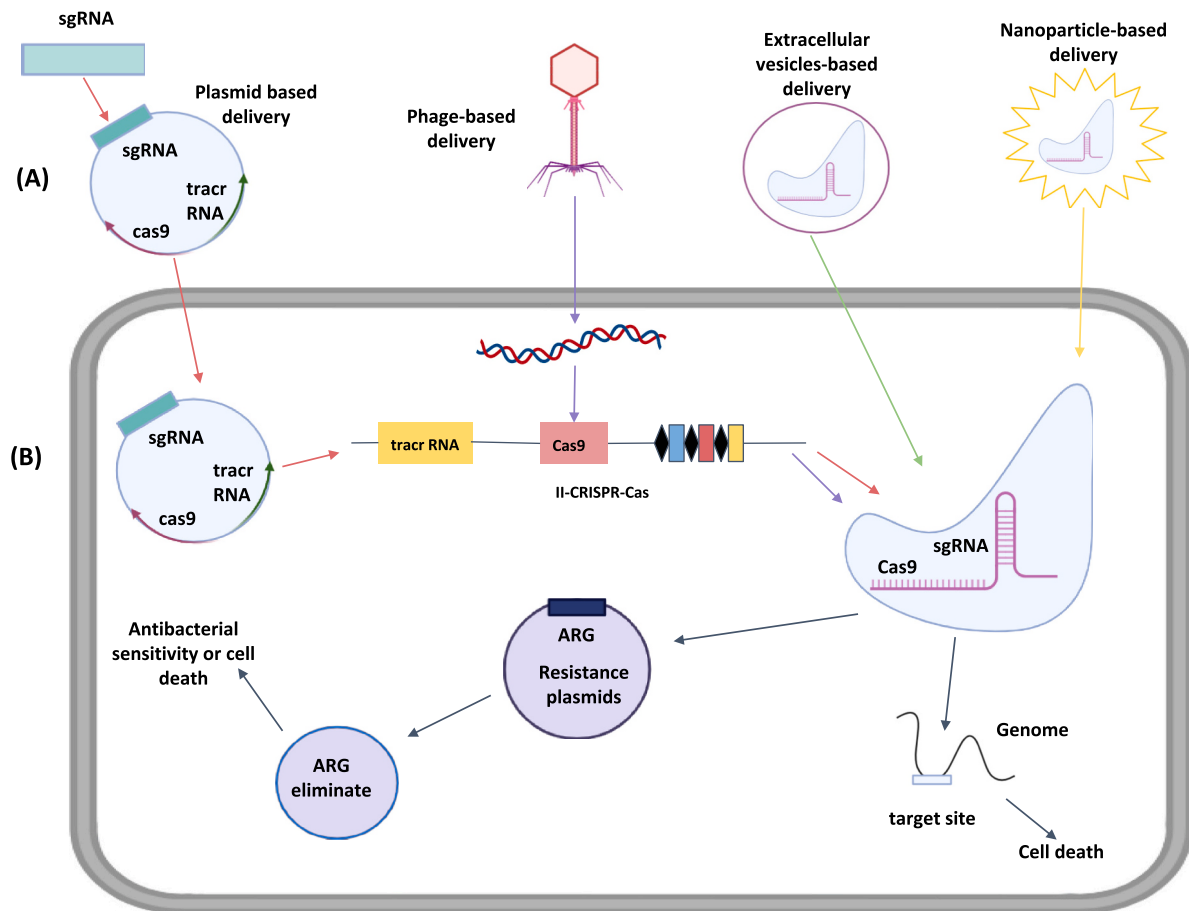
Fig. 3. (A) Methods for delivering CRISPR-Cas components: (1) Plasmid-based delivery: Design and synthesize sgRNA targeting the specific gene and ligate it into a plasmid vector containing Cas9; (2) phage-based delivery: Integrate the CRISPR-Cas system into the phage genome, which then acts as a vector for delivery. And (3) extracellular vesicles (EVs) and nanoparticle-based delivery: Package the Cas9 protein and sgRNA into a ribonucleoprotein (RNP) complex, which is then enclosed within EVs or nanoparticles. (B) Application of the CRISPR-Cas system in combating antibiotic resistance: When CRISPR-Cas systems are delivered into bacterial cells, they can target and eliminate antibiotic-resistant genes (ARGs) on plasmids, re-sensitizing the bacteria to antibacterial agents. Additionally, CRISPR-Cas exhibits strong bactericidal activity, causing cell death after recognizing the target ARG on the plasmid and the target site on the genome. Adapted from Tao et al. (2022).

4. Bioinformatics in ARG analysis

4.1. Role of bioinformatic tools in data processing and analysis from high-throughput studies

Bioinformatics is crucial for processing and analyzing data from high-throughput studies on ARGs, handling the vast amounts of data generated by large-scale sequencing technologies, and enabling the extraction, annotation, and interpretation of ARG-related information. Tools such as ARGs-OAP, ResFinder, and CARD (Comprehensive Antibiotic Resistance Database) are commonly used for these tasks (Table 4). ARGs-OAP facilitates the extraction and detailed annotation of ARG sequences from metagenomic datasets (Yang et al., 2013), while Res-Finder and CARD identify resistance genes in assembled whole-genome sequences, offering insights into their genetic context (Florensa et al., 2022; Alcock et al., 2023). Additionally, bioinformatic tools automate repetitive tasks, such as MG-RAST (Metagenomics Rapid Annotation using Subsystem Technology), which processes metagenomic data and provides functional annotations and taxonomic classifications (Keegan et al., 2016), and QIIME 2 (Quantitative Insights into Microbial Ecology), a pipeline for analyzing microbiome data that integrates ARG analysis with microbial community profiling (Estaki et al., 2020).

Several studies have demonstrated the utility of bioinformatic tools in ARG analysis (see Table 4). Forsberg et al. (2012) utilized metagenomic sequencing and bioinformatics to uncover a diverse array of

ARGs in soil microbiomes, highlighting the potential for environmental reservoirs of resistance genes. Another study by Zhu et al. (2017) employed bioinformatic pipelines to analyze ARGs in wastewater treatment plants, providing insights into the spread of resistance genes in aquatic environments. Specific bioinformatic pipelines and software packages are designed to streamline ARG analysis. For instance, Meta-PhlAn (Metagenomic Phylogenetic Analysis) offers a computational framework for profiling the composition of microbial communities and their associated ARGs (Segata et al., 2012). Another example is the AMR++ pipeline, which integrates multiple tools for comprehensive ARG detection and analysis from metagenomic data (Bonin et al., 2023).

4.2. Databases specifically designed for ARG identification and classification

Several widely used ARG databases support research on antibiotic resistance, including CARD, ResFinder, and ARG-ANNOT (Table 4). CARD (Comprehensive Antibiotic Resistance Database) provides a curated collection of over 5000 experimentally verified ARG sequences with detailed annotations on resistance mechanisms, gene families, and links to scientific literature. It also includes the Resistance Gene Identifier (RGI) tool for predicting ARGs from nucleotide sequences (Alcock et al., 2023; Jia et al., 2017). ResFinder, developed by the Center for Genomic Epidemiology, is a web-based tool that identifies antimicrobial resistance genes from bacterial whole-genome sequencing data, offering

Table 4
Bioinformatic tools and ARG databases are widely used in the research community.

	Website	Use	Reference
ARG databases			
CARD (Comprehensive Antibiotic Resistance Database)	https://card.mcmaster.ca/	Provides a curated collection of molecular sequences and associated data on antibiotic resistance genes.	Alcock et al. (2023)
ResFinder	http://genepi.food.dtu.dk/resfinder	Identifies acquired antibiotic resistance genes in bacterial genomes using whole-genome sequencing data.	Florensa et al. (2022)
ARDB (Antibiotic Resistance Genes Database)	https://ardb.cbcb.umd.edu/	Contains information about known antibiotic resistance genes and their associated proteins.	Liu and Pop (2009)
ARG-ANNOT (Antibiotic Resistance Gene-ANNOTation)	https://www.mediterranee-infection.com/acces-ressources/base-de-donnees/arg-annot-2/	Provides a comprehensive database for the annotation of antibiotic resistance genes.	Gupta et al. (2014)
PATRIC (Pathosystems Resource Integration Center)	https://www.patricbrc.org	Offers a variety of bioinformatics tools and databases, including those for studying antibiotic resistance genes.	Wattam et al. (2017)
DeepARG	https://bitbucket.org/gusphdproj/deeparg-ss/src/master/	Uses deep learning methods to predict antibiotic resistance genes from metagenomic data.	Arango-Argoty et al. (2018)
BacAnt	https://github.com/xthua/bacant	Comprehensive database and tool for the annotation of bacterial genomes, focusing on antimicrobial resistance genes, virulence factors, and other functional genes.	Hua et al. (2021)
AMRFinder	https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/AMRFinder/	Identifies antimicrobial resistance genes from genomic data, including point mutations associated with resistance.	Feldgarden et al. (2019)
AMRFinderPlus	https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/AMRFinder/	Extends AMRFinder capabilities by including additional detection for virulence factors and other resistance-associated genes.	Feldgarden et al. (2021)
MegaRes	https://www.meglab.org/megares/	Comprehensive database for annotated ARGs, tailored specifically for use in high-throughput sequencing experiments.	Bonin et al. (2023)
BacMet	http://bacmet.biomedicine.gu.se/	Provides information on antibacterial biocide and metal resistance genes. It includes experimentally verified and predicted antimicrobial resistance genes.	Pal et al. (2014)
Bioinformatic tools			
SPAdes (St. Petersburg genome assembler)	http://bioinf.spbau.ru/spades	Used for assembling genomes from short-read sequencing data, particularly effective for bacterial genomes.	Bankevich et al. (2012)
ARGs-OAP	https://galaxyproject.org/use/args-oap/	Used for the high-throughput analysis of ARGs in metagenomic data.	Yang et al. (2013)
ARESdb	https://ares-genetics.cloud/	Curated database of antimicrobial resistance elements, providing detailed annotations and resistance profiles.	Ferreira et al. (2020)
MG-RAST (Metagenomics Rapid Annotation using Subsystem Technology)	https://www.mg-rast.org/	Provides a comprehensive suite of tools for processing raw sequence data from environmental samples, allowing researchers to gain insights into the microbial communities present in those samples	Keegan et al. (2016)
QIIME2 (Quantitative Insights Into Microbial Ecology)	https://qiime2.org	Open-source software package that provides a comprehensive suite of tools for analyzing and interpreting microbial community data, primarily from high-throughput DNA sequencing techniques such as 16S rRNA gene sequencing	Estaki et al. (2020)
MetaX	http://metax.genomics.cn/	A tool for the comprehensive analysis of metabolomic data, including normalization, missing value imputation, and statistical analysis.	Wen et al. (2017)
MetaQuantome	https://github.com/galaxyproteomics/metaquantome	Designed for the quantitative analysis of metaproteomic data, facilitating the study of functional profiles of microbial communities.	Mehta et al. (2021)
Resfams	http://resfams.org/	Offers a curated library of protein families for identifying ARGs in metagenomic data. It uses hidden Markov models (HMMs) to detect resistance proteins.	Gibson et al. (2015)
ARIBA (Antibiotic Resistance Identification By Assembly)	https://github.com/sanger-pathogens/ariba	A tool designed for identifying antibiotic resistance genes in bacterial sequences. It can be used to detect ARGs by assembling and mapping short reads against a reference database.	Hunt et al. (2017)
RGI (Resistance Gene Identifier) tool	https://card.mcmaster.ca/analyze/rgi	A computational tool developed by the Comprehensive Antibiotic Resistance Database (CARD) team to predict antimicrobial resistance (AMR) genes in genomic and metagenomic data.	Jia et al. (2017)

a user-friendly interface and integrating diverse ARG datasets for comprehensive coverage (Florensa et al., 2022). Both CARD and Res-Finder are actively maintained as of 2024. ARG-ANNOT, a legacy database last updated in 2019, contains over 2000 ARG sequences for annotating and classifying resistance genes in bacterial genomes (Gupta et al., 2014).

5. Environmental monitoring methods

5.1. Biosensors for monitoring ARGs

Biosensors are analytical devices which encompass a biological recognition element along with a physio-chemical transducer used to detect and quantify specific analytes (Turner, 2013). Biosensors operate on the principle that the biological recognition element selectively interacts with the analyte providing a measurable signal proportional to the analyte concentration. This produced signal is transformed into a readable output having real time and sensitive detection capabilities. Biosensors are one of the main ways to detect ARGs as they are highly specific, sensitive, and provide rapid responses.

There are different types of biosensors. Optical biosensors are instrumental in ARG detection, tracking changes in light due to ARG-bioreceptor binding and providing precise, real-time data (Peltomaa et al., 2018). These sensors utilize various mechanisms such as fluorescence, surface plasmon resonance (SPR), and colorimetry to measure ARG interactions optically. Electrochemical biosensors are another

prevalent type, detecting ARGs through electrical response changes from binding events (Wang et al., 2023). They offer sensitivity and portability, making them suitable for in-field ARG surveillance. Types include amperometric, monitoring reaction-current changes, potentiometric, sensing voltage shifts, and impedimetric, detecting impedance variations due to ARG presence. Their cost-effectiveness and quick results align with environmental and clinical demands for ARG analysis. Piezoelectric biosensors present an alternative method by gauging ARG binding-induced mass changes, quantifiable through shifts in crystal oscillation frequency, which allows for direct, real-time, and label-free ARG quantification with high sensitivity (Tiquia-Arashiro, 2012; Zhao et al., 2013). These biosensor technologies collectively facilitate a comprehensive approach to ARG monitoring, providing detailed, dynamic insights into antibiotic resistance spread.

Recent advancements in biosensor technology have markedly improved the real-time monitoring of antibiotic resistance genes (ARGs) in aquatic environments. For instance, Mao et al. (2024) introduced a portable biosensor that enables real-time monitoring, simplifying on-site testing and reducing the need for extensive sample preparation (Fig. 4). Their potential for miniaturization allows for portable devices, while some biosensors can even detect multiple targets simultaneously, increasing efficiency. These advancements highlight the evolving landscape of biosensor technology, enhancing our ability to monitor and manage antibiotic resistance in aquatic settings effectively. Of course,

being able to detect ARGs in real time would be invaluable in minimizing the consequences that may follow the expression of ARGs. In another study the Evanescent wave Dual-color fluorescence Fiber-embedded Optofluidic Nanochip (EDFON) was created to directly detect the expression of ARGs in aquatic environments (Chen et al., 2024). The nanochip had a nano-biosensor embedded within it, which allows it to recognize the genes that express antibiotic resistance. In one instance, this technology was used to detect the antibiotic resistance gene MCR-1 with high sensitivity and specificity using hybridization chain reaction (Chen et al., 2024). This was an important showcase since MCR-1 is expressed after long-term exposure to sulfonamide that is used to treat bacterial infections (Chen et al., 2024). The quick and on-site detection allows for greater flexibility, especially for rapid expressions of antibiotic-resistant genes in bacterial communities.

While biosensors are promising for monitoring ARGs in aquatic ecosystems with high precision and in real-time (Turner, 2013), their adoption is hampered by several issues. Aquatic samples often contain substances, such as salts and organics that can interfere with results, leading to false positives or sensitivity losses (McKindles and Tiquia-Arashiro, 2012; Peltomaa et al., 2018). Additionally, complex sample processing required for effective detection can constrain their on-field utility (Wang et al., 2023). The absence of multiplexing limits simultaneous ARG detection across diverse microbial profiles (Zhao et al., 2013). Moreover, biosensors must be made more durable and stable for
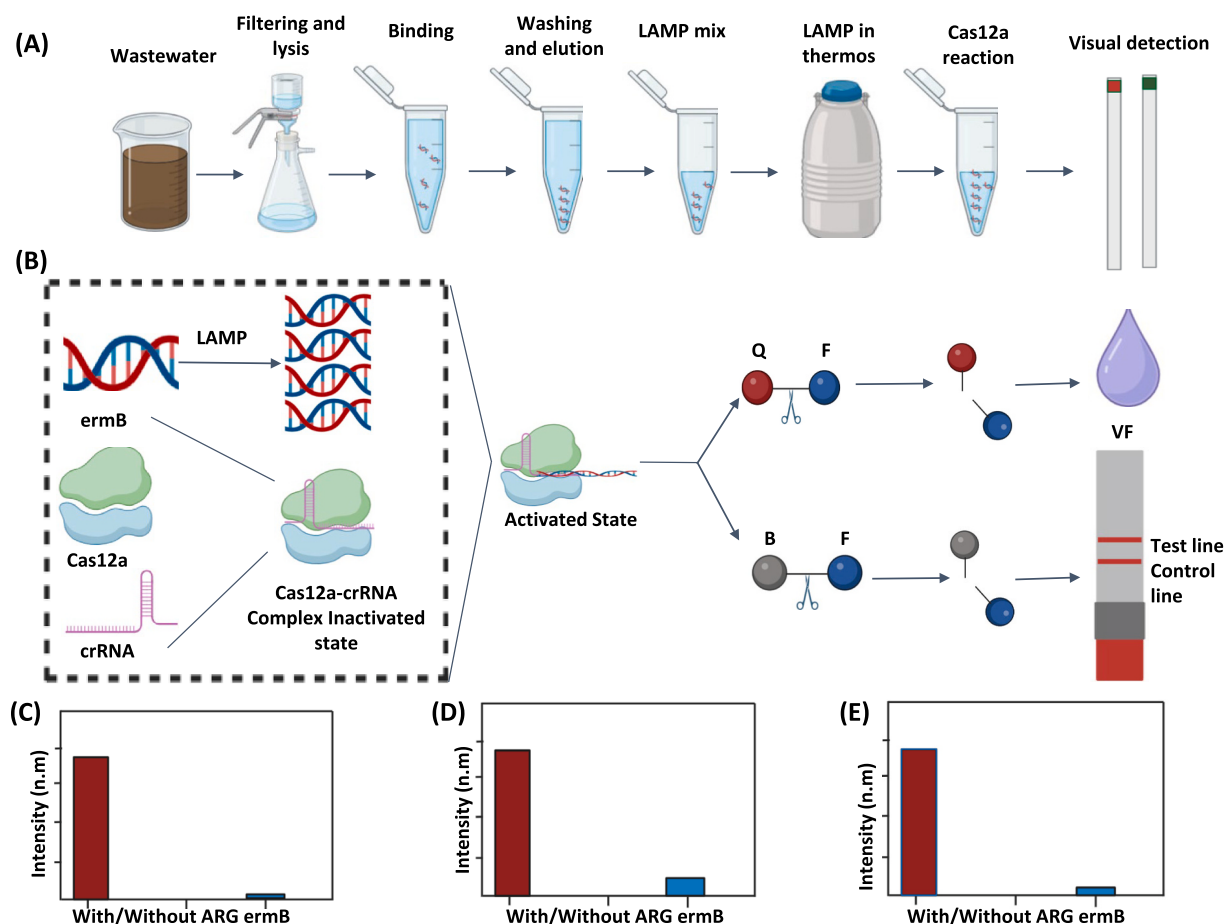
Fig. 4. Detection mechanism of the constructed biosensor for ARG *erm*B in wastewater. (A) The detection process of the biosensor includes filtering and lysing the sample, isolating target molecules with magnetic bead extraction, pre-amplifying the target DNA using LAMP, recognizing and transducing with the CRISPR/Cas12a system, and detecting the target DNA using fluorescence and a lateral flow device. (B) Cas12 crRNA specifically recognize and detect the *erm*B gene preamplified by LAMP. (C) The feasibility of the independent CRISPR/Cas12a-based analytical method was tested for ARG ermB at a concentration of 10 nM, while (D) the feasibility of the LAMP method was tested at a concentration of 50 pM. (E) Finally, the feasibility of the integrated portable CRISPR/Cas12a-based biosensor for detecting ARG ermB was also evaluated.
Adapted from Mao et al. (2024).

field conditions, while costs for setup and maintenance need reduction to improve access, especially in low-resource areas (Mao et al., 2024; Chen et al., 2024). Lastly, standardized protocols are needed to validate biosensor results and integrate data across studies for consistent monitoring outputs (Chen et al., 2024). Enhancing biosensors for ARG detection in aquatic systems involves creating devices resistant to interference from salts and organics, streamlining sample processing for better portability and on-site applications, and incorporating multiplexing to detect multiple ARGs simultaneously for a comprehensive analysis of resistance. Further, improving sensor stability, reducing costs, and adopting standardized protocols are essential to ensure dependable performance, especially in low-resource settings. These steps will bolster the effectiveness of biosensors in tracking environmental antibiotic resistance.

5.2. Geographic information systems (GIS)

The use of geospatial technologies in monitoring antibiotic resistance genes (ARGs) in aquatic ecosystems represents a strategic integration of technology and landscape ecology that can be used to understand the prevalence, transmission, and prevention of ARGs (Chique et al., 2019). GIS enables the creation of detailed maps that highlight the ARG hotspots, illustrating areas with high concentrations of ARGs. This spatial detail is invaluable for establishing a baseline of environmental ARG levels and tracking changes over time (Zhu, 2016). Researchers can use GIS to investigate the relationships between ARG prevalence and various environmental and anthropogenic factors. For example, spatial analysis can reveal possible correlations between ARG concentrations and various geographic factors, such as land use, proximity to human or animal populations, and the presence of medical or agricultural facilities (Van Boeckel et al., 2015; Chique et al., 2019). This aids in identifying critical areas for intervention and helps prioritize resource allocation efficiently. By integrating a diversity of ARG data with these geographic layers, GIS allows the identification and ranking of potential risk factors influencing the presence of ARGs in aquatic systems (Xu et al., 2023). This includes examining factors such as wastewater discharge points, agricultural runoff, and urban development, thereby providing a comprehensive understanding of the ARG landscape. This spatial correlation can be crucial for determining where intervention strategies might be most effective. Additionally, using GIS, researchers can develop predictive models that utilize environmental data to forecast the movement and proliferation patterns of ARGs. These models can simulate ARG dynamics in response to variables such as environmental gradients, hydrological changes, and human activities (Freire et al., 2024). By applying hydrological models and incorporating known behaviors of ARG transmission, GIS can forecast the future spread of ARGs in waterways, aiding in proactive management efforts. This approach provides a methodology for understanding and modeling the complex spread of antibiotic-resistant phenotypes in multi-drug environments and has the potential to enhance GIS-based environmental monitoring efforts for controlling ARG proliferation.

As an example, a recent study by Xu et al. (2023) provides a detailed analysis of antibiotic resistance genes (ARGs) in global oceanic environments, incorporating data from metagenomic (MetaG) and metatranscriptomic (MetaT) studies (Fig. 5). The geographic distribution of samples (Fig. 5A) spans diverse latitudes and longitudes, with orange points representing MetaG samples, yellow points for MetaT, and pink points indicating locations with both data types, enabling a broad-scale assessment of ARG diversity and abundance. The composition of ARG classes (Fig. 5B) reveals beta-lactams as the most dominant ARG type (394 occurrences), followed by multidrug resistance (126) and Aminoglycosides (97), with other classes like MLS, Glycopeptides, and Tetracycline also present, reflecting the widespread prevalence of resistance mechanisms. Fig. 5C and D explores ARG diversity and abundance relative to latitude, showing weak positive correlations, suggesting minor latitudinal influences alongside other significant factors. Fig. 5E

and F examines ARG diversity and abundance across oceanic depths, with surface layers (SRF) exhibiting significantly higher diversity than deeper layers, although abundance differences are not statistically significant. The data collectively highlight that ARG diversity is influenced by latitude and depth, with the surface layer's proximity to anthropogenic activities likely contributing to its higher diversity. The dominance of beta-lactam ARGs underscores their global prevalence and persistence in marine environments, emphasizing the need for further research on ARG dissemination in ocean ecosystems and their potential impact on human and environmental health. Thus, geospatial technologies offer significant benefits in monitoring antibiotic resistance genes (ARGs), including the integration of diverse datasets for a comprehensive understanding of ARG distribution and drivers, enhanced decision-making through maps and models that guide targeted interventions, and scalability for applications ranging from local to global studies. However, challenges remain, such as the need for high-resolution data, the complexity of integrating biological and environmental datasets, and uncertainties in predictive models. Overcoming these obstacles requires interdisciplinary collaboration among microbiologists, environmental scientists, and geospatial analysts.

5.3. Remote sensing technologies

Remote sensing technologies are emerging as invaluable tools for monitoring ARG and ARB monitoring across expansive aquatic ecosystems. These methods employ non-invasive techniques to gather extensive environmental data, which can reflect the presence of ARGs and ARBs (Khan et al., 2018; Callejas, 2023). Remote sensing encompasses a variety of technologies including satellite imagery, drone-based observations, and other aerial data collection methods. These tools can measure various water quality parameters such as turbidity, temperature, and chemical concentrations, which are often indicators of ARG prevalence (Hanlon et al., 2022; Sudriani et al., 2023; Lausch et al., 2024; Xie et al., 2024; Book et al., 2025). Remote sensing can cover large geographical areas, providing a synoptic view that is difficult to achieve with ground-based sampling alone, especially in vast and difficult-to-access aquatic ecosystems. Satellite and drone-based sensors can capture high-resolution spatial and temporal data, making it possible to monitor changes in water quality and the distribution of ARGs and ARBs over time. Specific parameters that can be measured include turbidity, where high levels can correlate with increased microbial activity, including ARGs (Hanlon et al., 2022); temperature, which influences bacterial growth and the spread of ARGs (Xie et al., 2024); and chemical concentrations, with the presence of certain chemicals promoting the proliferation of ARGs (Sudriani et al., 2023).

Remote sensing methods offer numerous benefits, including the ability to collect environmental data non-invasively, which allows for the monitoring of sensitive or protected ecosystems without causing harm (Khan et al., 2018; Callejas, 2023). These technologies also provide extensive coverage, efficiently gathering comprehensive data over large areas that would be time-consuming and costly to collect through traditional ground-based methods (Hanlon et al., 2022). The high-resolution data collected at both spatial and temporal scales enable detailed monitoring of environmental changes and ARG distributions (Lausch et al., 2024). Additionally, remote sensing data can be integrated with machine learning algorithms and GIS. For instance, identifying ARG resistance hotspots through remote sensing and machine learning can help in targeting specific areas for mitigation efforts (Cuadros et al., 2024). Similarly, the integration of remote sensing data with GIS allows for the visualization of complex relationships between environmental variables and ARG prevalence, offering deeper insights into the factors driving antibiotic resistance in aquatic ecosystems (Berglund, 2015). Over time, remote sensing proves to be more cost-effective compared to frequent and widespread traditional sampling (Xie et al., 2024), and some systems even offer near real-time data collection, allowing for timely responses to emerging environmental
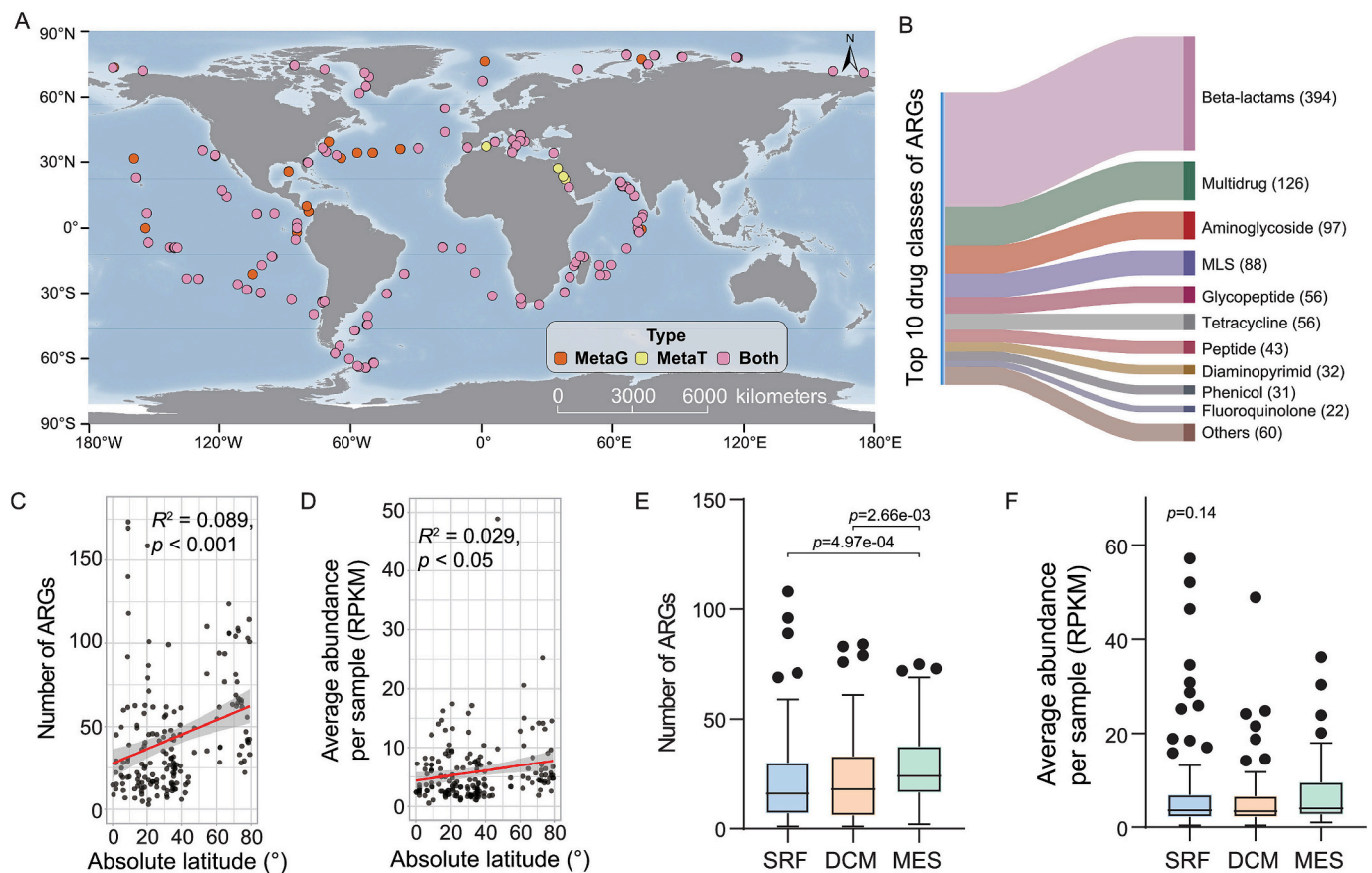
Fig. 5. Broad-spectrum ARG profiles in the global oceans. (A) The geographic distribution of samples is shown, with orange points indicating metagenomic (MetaG) samples, yellow points indicating metatranscriptomic (MetaT) samples, and pink points indicating paired metagenomic and metatranscriptomic samples. (B) The composition of the 10 most common antibiotic classes of ARGs is presented. (C) and (D) display the diversity and abundance of ARGs with latitude, with *p*-values indicating statistical significance (ANOVA F-test). (E) and (F) show the diversity and abundance of ARGs with depth.
Reprinted from Publication: Xu et al. (2023). A global atlas of marine antibiotic resistance genes and their expression. Water Res. 2023 Oct 1;244:120488. doi:10.1016/j.watres.2023.120488.

threats (Said and Khan, 2021).

However, the effective use of GIS in ARG monitoring faces several challenges. These include the need for high-quality data, accurately representing complex biological interactions within a digital medium, and the constant evolution of ARGs. Additionally, GIS analyses can be constrained by the resolution and accuracy of available spatial data sets and the sophistication of the models used to interpret those data. Remote sensing also has limitations, such as being an indirect method for ARG detection and requiring specialized expertise to process and interpret the data accurately.

6. Conclusions and future perspectives

This review underscores the urgent need for comprehensive strategies to manage antibiotic resistance genes (ARGs) in aquatic ecosystems. Human activities, including wastewater discharge and agricultural runoff, continue to exacerbate the spread of ARGs, emphasizing the importance of understanding and mitigating this issue. Advances in molecular techniques such as PCR, qPCR, metagenomics, and functional genomics have significantly improved ARG detection and characterization, though challenges like complex sample processing and reliance on bioinformatics resources persist. Future research should aim to track ARG dynamics over time and integrate transcriptional, proteomic, and metabolic insights alongside genomic data. Overcoming data processing and analysis challenges will require advancements in algorithms, protocol standardization, and comprehensive reference databases.

Biosensor technologies hold promise for real-time, sensitive ARG detection. Recent innovations focus on addressing challenges related to interference, stability, and multiplexing, aiming for devices that are durable, cost-effective, and functional under diverse environmental conditions. Establishing a global framework for standardized data collection and interpretation will be crucial to assessing the progression of antibiotic resistance and its ecological and public health impacts. This can be supported by investments in cloud computing and artificial intelligence to enhance predictive modeling and ARG tracking capabilities.

The integration of large-scale datasets requires continuous refinement of bioinformatics tools, updated databases, and real-time monitoring systems, such as biosensors. Additionally, Geographic Information Systems (GIS) and remote sensing technologies offer significant potential for monitoring ARGs, though they must address challenges like data quality, environmental variability, and technical constraints to provide actionable insights.

Future research directions include:

1. Advancing CRISPR Platforms: Developing CRISPR-based systems that operate effectively in diverse aquatic conditions and incorporating multiplexing to track multiple ARGs simultaneously, enhancing monitoring efficiency.
2. Machine Learning Integration: Refining machine learning models to predict ARG presence and levels from complex environmental samples, with potential for integrating omics data to unravel resistance mechanisms.

3. Expanding Omics Technologies: Using omics approaches to explore ARG diversity and activity within microbial communities, potentially achieving single-cell resolution to uncover complex resistance pathways.
4. Enhancing GIS and Big Data Analytics: Leveraging high-resolution environmental data and integrating GIS with machine learning and big data analytics to improve predictive capabilities, enabling proactive management of ARG spread.
5. Deploying Real-Time Remote Sensing: Developing remote sensing systems for immediate water quality assessments, enabling timely interventions to curb ARG proliferation.

As global attention to antibiotic resistance grows, these innovative approaches will be critical in advancing our ability to monitor, manage, and mitigate ARG impacts on aquatic ecosystems and public health.

CRediT authorship contribution statement

Zainab N. Nassereddine: Writing – review & editing, Writing – original draft, Validation, Formal analysis, Data curation. **Somie D. Opara:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis. **Oliver A. Coutinho:** Writing – review & editing, Writing – original draft, Validation, Formal analysis, Data curation. **Florent Qyteti:** Writing – review & editing, Writing – original draft, Validation, Formal analysis. **Reeghan Book:** Writing – review & editing, Writing – original draft, Validation, Formal analysis. **Matthew P. Heinicke:** Writing – review & editing, Writing – original draft. **Jacob Napieralski:** Writing – review & editing, Writing – original draft. **Sonia M. Tiquia-Arashiro:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

Acknowledgments

Data availability

No data was used for the research described in the article.

References

Abdi, G., et al., 2024. Unveiling the dynamic role of bioinformatics in automation for efficient and accurate data processing and interpretation. In: Singh, V., Kumar, A. (Eds.), Advances in Bioinformatics. Springer, Singapore. https://doi.org/10.1007/978-981-99-8401-5_15.

Abraham, E.P., Chain, E., 1988 Jul-Aug. An enzyme from bacteria able to destroy penicillin. 1940. Rev. Infect. Dis. 10 (4), 677–678 (PMID: 3055168).

Ahmad, I., Siddiqui, S.A., Khan, S.A., Ali, A., Chaudhary, N., 2024. Chapter 6.6 - cultural and molecular approaches to analyse antimicrobial resistant bacteria from environmental samples. In: Das, Surajit, Dash, Hirak Ranjan (Eds.), Microbial Diversity in the Genomic Era, Second edition. Academic Press, pp. 759–776. https://doi.org/10.1016/B978-0-443-13320-6.00014-7.

Alam, M.-U., Ferdous, S., Ercumen, A., et al., 2021. Effective treatment strategies for the removal of antibiotic-resistant bacteria, antibiotic-resistance genes, and antibiotic residues in the effluent from wastewater treatment plants receiving municipal, hospital, and domestic wastewater: protocol for a systematic review. JMIR Research Protocols 10 (11), e33365. https://doi.org/10.2196/33365.

Alcock, B.P., Huynh, W., Chalil, R., Smith, K.W., Raphenya, A.R., et al., 2023. CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. Nucleic Acids Res. 51 (D1), D690–D699. https://doi.org/10.1093/nar/gkac920 (Jan 6).

Amos, G.C., Zhang, L., Hawkey, P.M., Gaze, W.H., Wellington, E.M., 2014. Functional metagenomic analysis reveals rivers are a reservoir for diverse antibiotic resistance genes. Vet. Microbiol. 171 (3–4), 441–447. https://doi.org/10.1016/j.vetmic.2014.02.017 (Jul 16).

Arango-Argoty, G., Garner, E., Pruden, A., et al., 2018. DeepARG: a deep learning approach for predicting antibiotic resistance genes from metagenomic data. Microbiome 6 (1), 23. https://doi.org/10.1186/s40168-018-0401-z.

Armstrong, J.L., Shigeno, D.S., Calomiris, J.J., Seidler, R.J., 1981. Antibiotic-resistant bacteria in drinking water. Appl. Environ. Microbiol. 42 (2), 277–283. https://doi.org/10.1128/aem.42.2.277-283.1981.

Bankevich, A., Nurk, S., Antipov, D., et al., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19 (5), 455–477. https://doi.org/10.1089/cmb.2012.0021 (May).

Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45 (4), 493–496 (Apr, PMID: 5325707).

Begmatov, S., Beletsky, A.V., Dorofeev, A.G., et al., 2024. Metagenomic insights into the wastewater resistome before and after purification at large-scale wastewater treatment plants in the Moscow city. Sci. Rep. 14 (1), 6349. https://doi.org/10.1038/s41598-024-56870-0.

Bell, J.B., Macrae, W.R., Elliott, G.E., 1980. Incidence of R factors in coliform, fecal coliform, and Salmonella populations of the Red River in Canada. Appl. Environ. Microbiol. 40 (3), 486–491. https://doi.org/10.1128/aem.40.3.486-491.1980.

Bengtsson-Palme, J., Hammarén, R., Pal, C., et al., 2016. Elucidating selection processes for antibiotic resistance in sewage treatment plants using metagenomics. Sci. Total Environ. 572, 697–712. https://doi.org/10.1016/j.scitotenv.2016.06.228.

Berglund, B., 2015. Environmental dissemination of antibiotic resistance genes and correlation to anthropogenic contamination with antibiotics. Infect Ecol Epidemiol. (5), 28564. https://doi.org/10.3402/iee.v5.28564 (Sep 8, PMID: 26356096; PMCID: PMC4565060).

Blainey, P.C., 2013. The future is now: single-cell genomics of bacteria and archaea. FEMS Microbiol. Rev. 37 (3), 407–427. https://doi.org/10.1111/1574-6976.12015.

Bonin, N., Doster, E., Worley, H., et al., 2023. MEGARes and AMR++, v3.0: an updated comprehensive database of antimicrobial resistance determinants and an improved software pipeline for classification using high-throughput sequencing. Nucleic Acids Res. 51 (D1), D744–D752. https://doi.org/10.1093/nar/gkac1047 (Jan 6).

Bonnet, M., Lagier, J.C., Raoult, D., Khelaifia, S., 2020. Bacterial culture through selective and non-selective conditions: the evolution of Culture Media in Clinical Microbiology. New Microbes and New Infections 34, 100622. https://doi.org/10.1016/j.nmni.2019.100622.

Book, R., Qyteti, F., Nassereddine, Z., Tiquia-Arashiro, S., 2025. Integrated approaches to tracking antibiotic resistance in aquatic ecosystems. In: Watershed Microbiology: Biodiversity Composition, Ecological Function, and Technological Advances. Springer International Publishing (in press).

Bowman, N., Patel, P., Xu, W., et al., 2018. Enrichment and isolation of Pb-resistant bacteria from Saint Clair River sediments and their potential for Pb removal in aqueous solutions. Appl. Microbiol. Biotechnol. 102, 2391–2398. https://doi.org/10.1007/s00253-018-8772-4.

Buffet-Bataillon, S., Tattevin, P., Maillard, J.Y., Bonnaure-Mallet, M., Jolivet-Gougeon, A., 2016. Efflux pump induction by quaternary ammonium compounds and fluoroquinolone resistance in bacteria. Future Microbiol. 11 (1), 81–92. https://doi.org/10.2217/fmb.15.131.

Callejas, I., 2023. Emerging Techniques in Coastal Water Quality in the US and Belize: Remote Sensing and Metagenomics. University of California, Los Angeles (dissertation, ProQuest Dissertations & Theses; 30525616).

Camacho, D.M., Collins, K.M., Powers, R.K., et al., 2018. Next-generation machine learning for biological networks. Cell 173 (7), 1581–1592. https://doi.org/10.1016/j.cell.2018.05.015.

Caporaso, J.G., Lauber, C.L., Walters, et al., 2012. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. 108 (Supplement 1), 4516–4522. https://doi.org/10.1073/pnas.1000080107.

Carrasco-Acosta, M., Garcia-Jimenez, P., 2024. Development of multiplex RT qPCR assays for simultaneous detection and quantification of faecal indicator bacteria in bathing recreational waters. Microorganisms 12 (6), 1223. https://doi.org/10.3390/microorganisms12061223.

Catalfano, B., Chamlee, Y., Khamis, V., Kasperek, D., Parmenter, E., Schittenhelm, R., Nassereddine, Z., Tiquia-Arashiro, S., 2025. Diverse aminoglycoside gene cassettes (*aad*A1, *aad*B, and *aac*A4) in class 1 integrons in Escherichia coli strains isolated from three urban watersheds. In: Watershed Microbiology: Biodiversity Composition, Ecological Function, and Technological Advances. Springer International Publishing (In press).

Chamlee, Y., Farinas, J., Pagnucco, G., Shuler, C., Zielinkski, E., Sweetapple, D., Hammoud, Z., Tiquia-Arashiro, S., 2025. Metal-microbe interactions in urban watersheds: s study of siderophore production. In: Watershed Microbiology: Biodiversity Composition, Ecological Function, and Technological Advances. Springer International Publishing (in press).

Chen, B., Yang, Y., Liang, X., Yu, K., Zhang, T., Li, X., 2013. Metagenomic profiles of antibiotic resistance genes (ARGs) between human impacted estuary and deep-ocean sediments. Environ. Int. 61, 1–10. https://doi.org/10.1021/es403818e.

Chen, H., Jing, L., Yao, Z., Meng, F., Teng, Y., 2019a. Prevalence, source and risk of antibiotic resistance genes in the sediments of Lake Tai (China) deciphered by

metagenomic assembly: a comparison with other global lakes. Environ. Int. 127, 267–275. https://doi.org/10.1016/j.envint.2019.03.048 (Jun).

Chen, H., Bai, X., Jing, L., Chen, R., Teng, Y., 2019b. Characterization of antibiotic resistance genes in the sediments of an urban river revealed by comparative metagenomics analysis. Sci. Total Environ. (653), 1513–1521. https://doi.org/10.1016/j.scitotenv.2018.11.052 (Feb 25).

Chen, D., Xu, W., Lu, Y., Zhuo, Y., Ji, T., Long, F., 2024. Rapid and sensitive parallel on-site detection of antibiotics and resistance genes in aquatic environments using evanescent wave dual-color fluorescence fiber-embedded OPTOFLUIDIC nanochip. Biosens. Bioelectron. 257, 116281. https://doi.org/10.1016/j.bios.2024.116281.

Chique, C., Cullinan, J., Hooban, B., Morris, D., 2019. Mapping and analysing potential sources and transmission routes of antimicrobial resistant organisms in the environment using geographic information systems—an exploratory study. Antibiotics 8 (1), 16. https://doi.org/10.3390/antibiotics8010016.

Cuadros, D.F., Chen, X., Li, J., Omori, R., Musuka, G., 2024. Advancing public health surveillance: integrating modeling and GIS in the wastewater-based epidemiology of viruses, a narrative review. Pathogens 13 (8), 685. https://doi.org/10.3390/pathogens13080685.

Davis, B.C., Brown, C., Gupta, S., Calarco, J., et al., 2023. Recommendations for the use of metagenomics for routine monitoring of antibiotic resistance in wastewater and impacted aquatic environments. Crit. Rev. Environ. Sci. Technol. 53 (19), 1731–1756. https://doi.org/10.1080/10643389.2023.2181620.

Daw Elbait, G., Daou, M., Abuoudah, M., et al., 2024. Comparison of qPCR and metagenomic sequencing methods for quantifying antibiotic resistance genes in wastewater. PLoS One 19 (4), e0298325. https://doi.org/10.1371/journal.pone.0298325 (Apr 5).

Demin, K., Prazdnova, E., Kulikov, M., et al., 2024. Alternative agar substitutes for culturing unculturable microorganisms. Arch. Microbiol. 206, 405. https://doi.org/10.1007/s00203-024-04139-5.

van Dijk, E.L., Jaszczyszyn, Y., Naquin, D., Thermes, C., 2018. The third revolution in sequencing technology. Trends Genet. 34 (9), 666–681. https://doi.org/10.1016/j.tig.2018.05.008 (Sep).

Eduardo-Correia, B., Morales-Filloy, H., Abad, J.P., 2020. Bacteria from the multi-contaminated Tinto River Estuary (SW, Spain) show high multi-resistance to antibiotics and point to Paenibacillus spp. as antibiotic-resistance-dissemination players. Front. Microbiol. (10), 3071. https://doi.org/10.3389/fmicb.2019.03071 (Jan 10).

Eisen, J.A., 2015. Environmental shotgun sequencing: its potential and challenges for studying the hidden world of microbes. PLoS Biol. 5 (3), e82. https://doi.org/10.1371/journal.pbio.0050082.

Estaki, M., Jiang, L., Bokulich, N.A., McDonald, D., et al., 2020. QIIME 2 enables comprehensive end-to-end analysis of diverse microbiome data and comparative studies with publicly available data. Curr. Protoc. Bioinformatics 70 (1), e100. https://doi.org/10.1002/cpbi.100 (Jun).

Feldgarden, M., Brover, V., Haft, D.H., et al., 2019. Validating the AMRFinder Tool and Resistance Gene Database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. Antimicrob. Agents Chemother. 63 (11), e00483-19. https://doi.org/10.1128/AAC.00483-19 (Oct 22, Erratum in: Antimicrob Agents Chemother. 2020 Mar 24;64(4): e00361-20. doi:10.1128/AAC.00361-20).

Feldgarden, M., Brover, V., Gonzalez-Escalona, N., Frye, J.G., et al., 2021. AMRFinderPlus and the Reference Gene Catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. Sci. Rep. 11 (1), 12728. https://doi.org/10.1038/s41598-021-91456-0 (Jun 16).

Ferrario, C., Alessandri, G., Mancabelli, et al., 2017. Untangling the cecal microbiota of feral chickens by culturomic and metagenomic analyses. Environ. Microbiol. 19 (11), 4771–4783. https://doi.org/10.1111/1462-2920.13943.

Ferraro, G.B., Bonomo, C., Brandtner, D., et al., 2024. Characterisation of microbial communities and quantification of antibiotic resistance genes in Italian wastewater treatment plants using 16S rRNA sequencing and digital PCR. Sci. Total Environ. 933, 173217. https://doi.org/10.1016/j.scitotenv.2024.173217.

Ferreira, I., Beisken, S., Lueftinger, L., Weinmaier, T., Klein, M., Bacher, J., Patel, R., von Haeseler, A., Posch, A.E., 2020. Species Identification and Antibiotic Resistance Prediction by Analysis of Whole-Genome Sequence Data by Use of ARESdb: an Analysis of Isolates from the Unyvero Lower Respiratory Tract Infection Trial. J. Clin. Microbiol. 58 (7), e00273–20. https://doi.org/10.1128/JCM.00273-20.

Florensa, A.F., Kaas, R.S., Clausen, P.T.L.C., et al., 2022. ResFinder - an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. Microb Genom. 8 (1), 000748. https://doi.org/10.1099/mgen.0.000748 (Jan).

Forsberg, K. J., Reyes, A., Wang, B., Selleck, E. M., Sommer, M. O., & Dantas, G. (2012). The shared antibiotic resistome of soil bacteria and human pathogens. Science, 337 (6098), 1107–1111. doi:https://doi.org/10.1126/science.1220761.

Fredriksen, S., de Warle, S., van Baarlen, P., et al., 2023. Resistome expansion in disease-associated human gut microbiomes. Microbiome 11, 166. https://doi.org/10.1186/s40168-023-01610-1.

Freire, T.F.A., Hu, Z., Wood, K.B., Gjini, E., 2024. Modeling spatial evolution of multi-drug resistance under drug environmental gradients. PLoS Comput. Biol. 20 (5), e1012098. https://doi.org/10.1371/journal.pcbi.1012098 (May 31).

Galhano, B.S., Ferrari, R.G., Panzenhagen, et al., 2021. Antimicrobial resistance gene detection methods for bacteria in animal-based foods: a brief review of highlights and advantages. Microorganisms 9 (5), 923. https://doi.org/10.3390/microorganisms9050923.

Gholizadeh, P., Köse, Ş, Dao, S., Ganbarov, K., Tanomand, A., Dal, T., Aghazadeh, M., Ghotaslou, R., Ahangarzadeh Rezaee, M., Yousefi, B., Samadi Kafil, H., 2020. How

CRISPR-Cas System Could Be Used to Combat Antimicrobial Resistance. Infect. Drug Resist. 13, 1111–1121. https://doi.org/10.2147/IDR.S247271.

Gibson, M.K., Forsberg, K.J., Dantas, G., 2015. Improved annotation of antibiotic resistance determinants reveals microbial resistomes cluster by ecology. ISME J. 9 (1), 207–216. https://doi.org/10.1038/ismej.2014.106.

Givens, C.E., Kolpin, D.W., Hubbard, L.E., et al., 2023. Simultaneous stream assessment of antibiotics, bacteria, antibiotic resistant bacteria, and antibiotic resistance genes in an agricultural region of the United States. Sci. Total Environ. (904), 166753. https://doi.org/10.1016/j.scitotenv.2023.166753 (Dec 15).

Goodwin, S., McPherson, J.D., McCombie, W.R., 2016. Coming of age: ten years of next-generation sequencing technologies. Nat. Rev. Genet. 17 (6), 333–351. https://doi.org/10.1038/nrg.2016.49.

Gupta, S.K., Padmanabhan, B.R., Diene, S.M., et al., 2014. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob. Agents Chemother. 58 (1), 212–220. https://doi.org/10.1128/aac.01310-13.

Gupta, S.S., Hamza, K.H.N., Sones, C.L., et al., 2024. The CRISPR/Cas system as an antimicrobial resistance strategy in aquatic ecosystems. Funct. Integr. Genomics 24, 110. https://doi.org/10.1007/s10142-024-01362-7.

Hanlon, R., Jacquemin, S.J., Birbeck, J.A., Westrick, J.A., et al., 2022. Drone-based water sampling and characterization of three freshwater harmful algal blooms in the United States. Front. Remote Sens. 3, 949052. https://doi.org/10.3389/frsen.2022.949052.

Hassan, M.M., van Vliet, A.H.M., Higgins, O., et al., 2023. Rapid culture-independent loop-mediated isothermal amplification detection of antimicrobial resistance markers from environmental water samples. Microb. Biotechnol. 16 (5), 977–989. https://doi.org/10.1111/1751-7915.14227 (May).

Hua, X., Liang, Q., Deng, M., He, J., Wang, M., et al., 2021. BacAnt: a combination annotation server for bacterial DNA sequences to identify antibiotic resistance genes, integrons, and transposable elements. Front. Microbiol. 12, 649969. https://doi.org/10.3389/fmicb.2021.649969.

Huggett, J.F., O'Grady, J., Bustin, S., 2015. qPCR, dPCR, NGS - a journey. Biomol Detect Quantif. (3), A1–A5. https://doi.org/10.1016/j.bdq.2015.01.001 (Jan 15).

Hunt, M., Mather, A.E., Sánchez-Busó, L., Page, A.J., Parkhill, J., Keane, J.A., Harris, S. R., 2017. ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. Microb Genom. 3 (10), e000131. https://doi.org/10.1099/mgen.0.000131 (Sep 4).

Idelevich, E.A., Becker, K., 2019. How to accelerate antimicrobial susceptibility testing. Clin. Microbiol. Infect. 25 (11), 1347–1355. https://doi.org/10.1016/j.cmi.2019.04.025 (Nov).

Jia, B., et al., 2017. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res. 45 (D1), D566–D573. https://doi.org/10.1093/nar/gkw1004.

Kassem, A., Abbas, L., Coutinho, O., Opara, S., Najaf, H., et al., 2023. Applications of Fourier Transform-Infrared spectroscopy in microbial cell biology and environmental microbiology: advances, challenges, and future perspectives. Front. Microbiol. 14, 1304081. https://doi.org/10.3389/fmicb.2023.1304081.

Kasuga, I., Nagasawa, K., Suzuki, M., Kurisu, F., Furumai, H., 2022. High-throughput screening of antimicrobial resistance genes and their association with class 1 integrons in urban rivers in Japan. Front. Environ. Sci. 10, 825372. https://doi.org/10.3389/fenvs.2022.825372.

Keegan, K.P., Glass, E.M., Meyer, F., 2016. MG-RAST, a metagenomics service for analysis of microbial community structure and function. Methods Mol. Biol. 1399, 207–233. https://doi.org/10.1007/978-1-4939-3369-3_13.

Keenum, I., Liguori, K., Calarco, J., et al., 2022. A framework for standardized qPCR-targets and protocols for quantifying antibiotic resistance in surface water, recycled water and wastewater. Crit. Rev. Environ. Sci. Technol. 52 (24), 4395–4419. https://doi.org/10.1080/10643389.2021.2024739.

Khan, F.U., et al., 2018. Remote sensing technologies for enhancing forest inventories: a review. Environ. Monit. Assess. 190 (9), 1–23. https://doi.org/10.1080/07038992.2016.1207484.

Kristiansson, E., Fick, J., Janzon, A., Grabic, R., et al., 2011. Pyrosequencing of antibiotic-contaminated river sediments reveals high levels of resistance and gene transfer elements. PLoS One 6 (2), e17038. https://doi.org/10.1371/journal.pone.0017038 (Feb 16).

Lausch, A., Bannehr, L., Berger, S.A., et al., 2024. Monitoring water diversity and water quality with remote sensing and traits. Remote Sens. 16 (13), 2425. https://doi.org/10.3390/rs16132425.

Levy, S.B., FitzGerald, G.B., Macone, A.B., 1976. Spread of antibiotic-resistant plasmids from chicken to chicken and from chicken to man. Nature 260 (5546), 40–42. https://doi.org/10.1038/260040a0 (Mar 4).

Lien, L.T.Q., Lan, P.T., Chuc, N.T.K., et al., 2017. Antibiotic resistance and antibiotic resistance genes in *Escherichia coli* isolates from hospital wastewater in Vietnam. Int. J. Environ. Res. Public Health 14 (7), 699. https://doi.org/10.3390/ijerph14070699.

Lino, C.A., Harper, J.C., Carney, J.P., et al., 2018. Delivering CRISPR: a review of the challenges and approaches. Drug Deliv. 25 (1), 1234–1257. https://doi.org/10.1080/10717544.2018.1474964.

Liu, B., Pop, M., 2009. ARDB—Antibiotic Resistance Genes Database. Nucleic Acids Res. 37 (Database issue), D443–D447. https://doi.org/10.1093/nar/gkn656 (Jan).

Liu, Z., Klümper, U., Liu, Y., Yang, Y., et al., 2019. Metagenomic and metatranscriptomic analyses reveal activity and hosts of antibiotic resistance genes in activated sludge. Environ. Int. 129, 208–220. https://doi.org/10.1016/j.envint.2019.05.036 (Aug).

Liu, S., Jørgensen, J.T., Ljungman, P., et al., 2021. Long-term exposure to low-level air pollution and incidence of chronic obstructive pulmonary disease: the ELAPSE project. Environ. Int. 146, 106267. https://doi.org/10.1016/j.envint.2020.106267.

Long, T.F., Zhou, S.Y., Huang, Z.L., Li, G., et al., 2024. Innovative delivery system combining CRISPR-Cas12f for combatting antimicrobial resistance in gram-negative Bacteria. ACS Synth. Biol. 13 (6), 1831–1841. https://doi.org/10.1021/acssynbio.4c00112 (Jun 21).

Louca, S., Parfrey, L.W., Doebeli, M., 2016. Decoupling function and taxonomy in the global ocean microbiome. Science 353 (6305), 1272–1277. https://doi.org/10.1126/science.aaf4507 (Sep 16).

Luo, Y., Mao, D., Rysz, M., Zhou, Q., et al., 2010. Trends in antibiotic resistance genes occurrence in the Haihe River. China. Environ Sci Technol. 44 (19), 7220–7225. https://doi.org/10.1021/es100233w (Oct 1).

Maestre-Carballa, L., Navarro-López, V., Martinez-Garcia, M., 2024. City-scale monitoring of antibiotic resistance genes by digital PCR and metagenomics. Environmental Microbiome 19, 16. https://doi.org/10.1186/s40793-024-00557-6.

Magnano San Lio, R., Favara, G., Maugeri, A., Barchitta, M., Agodi, A., 2023. How antimicrobial resistance is linked to climate change: an overview of two intertwined global challenges. Int. J. Environ. Res. Public Health 20 (3), 1681. https://doi.org/10.3390/ijerph20031681.

Manaia, C.M., Rocha, J., Scaccia, N., 2018. Antibiotic resistance in urban aquatic environments: can it be controlled? Appl. Microbiol. Biotechnol. 102 (2), 783–790. https://doi.org/10.1007/s00253-015-7202-0.

Mao, K., Zhang, H., Ran, F., Cao, H., Feng, R., Du, W., Li, X., Yang, Z., 2024. Portable biosensor combining CRISPR/Cas12a and loop-mediated isothermal amplification for antibiotic resistance gene *erm*B in wastewater. J. Hazard. Mater. (462), 132793. https://doi.org/10.1016/j.jhazmat.2023.132793 (Jan 15).

Marano, R.B.M., Fernandes, T., Manaia, C.M., Nunes, O., et al., 2020. A global multinational survey of cefotaxime-resistant coliforms in urban wastewater treatment plants. Environ. Int. 144, 106035. https://doi.org/10.1016/j.envint.2020.106035 (Nov).

Mark, D., Haeberle, S., Roth, G., et al., 2010. Microfluidic Lab-on-a-Chip platforms: requirements, characteristics and applications. In: Kakaç, S., Kosoy, B., Li, D., Pramuanjaroenkij, A. (Eds.), Microfluidics Based Microsystems, NATO Science for Peace and Security Series A: Chemistry and Biology. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-9029-4_17.

McKindles, K.M., Tiquia-Arashiro, S.M., 2012. Functional gene arrays for analysis of microbial communities on ocean platform. In: Tiquia-Arashiro, S. (Ed.), Molecular Biological Technologies for Ocean Sensing. Springer Protocols Handbooks. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-61779-915-0_9.

Mehta, S., Kumar, P., Crane, M., Johnson, J.E., et al., 2021. Updates on metaQuantome software for quantitative metaproteomics. J. Proteome Res. 20 (4), 2130–2137. https://doi.org/10.1021/acs.jproteome.0c00960 (Apr 2).

Mu, X., Nakano, R., Nakano, A., Ubagai, T., et al., 2016. Loop-mediated isothermal amplification: rapid and sensitive detection of the antibiotic resistance gene ISAba1-blaOXA-51-like in *Acinetobacter baumannii*. J. Microbiol. Methods 121, 36–40. https://doi.org/10.1016/j.mimet.2015.12.011.

Munir, M., Wong, K., Xagoraraki, I., 2011. Release of antibiotic-resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan. Water Res. 45 (2), 681–693. https://doi.org/10.1016/j.watres.2010.08.033 (Jan).

Muziasari, W.I., Pärnänen, K., Johnson, T.A., et al., 2016. Aquaculture changes the profile of antibiotic resistance and mobile genetic element associated genes in Baltic Sea sediments. FEMS Microbiol. Ecol. 92 (4), fiw052. https://doi.org/10.1093/femsec/fiw052.

Ng, C., Tay, M., Tan, B., Le, T.-H., Haller, L., et al., 2017. Characterization of metagenomes in urban aquatic compartments reveals high prevalence of clinically relevant antibiotic resistance genes in wastewaters. Front. Microbiol. 8, 2200. https://doi.org/10.3389/fmicb.2017.02200.

Nguyen, S, Ala F, Cardwell C, Cai D, McKindles KM, Lotvola A, Hodges S, Deng, Y. and Tiquia-Arashiro, S.M. (2013) Isolation and screening of carboxydotrophs isolated from composts and their potential for butanol synthesis. Environ. Technol. 34: 1995–2007. Doi:https://doi.org/10.1080/09593330.2013.795987.

Nnadozie, C.F., Odume, O.N., 2019. Freshwater environments as reservoirs of antibiotic-resistant bacteria and their role in the dissemination of antibiotic resistance genes. Environmental Pollution (Barking, Essex: 1987) 254 (Pt B), 113067. https://doi.org/10.1016/j.envpol.2019.113067.

Oest, A., Alsaffar, A., Fenner, J., Azzopardi, D., Tiquia-Arashiro, S.M., 2018. Patterns of change in metabolic capabilities of sediment microbial communities in river and Lake ecosystems. Int J Microbiol. (2018), 6234931. https://doi.org/10.1155/2018/6234931 (May 27).

Opara, S., Coutinho, O., Pagnucco, G., Shuler, C., Saab, R., Rubley, E., Kassem, A., Tiquia-Arashiro, S., 2025. Biosorption dynamics in bi-metal solutions by bacterial strains isolated from an urban watershed. In: Watershed Microbiology: Biodiversity Composition, Ecological Function, and Technological Advances. Springer International Publishing (In press).

Ordine, J.V.W., de Souza, G.M., Tamasco, G., et al., 2023. Metagenomic insights for antimicrobial resistance surveillance in soils with different land uses in Brazil. Antibiotics 12 (2), 334. https://doi.org/10.3390/antibiotics12020334.

Pagnucco, G., Overfield, D., Chamlee, Y., Fortuna, A., et al., 2023. Metal tolerance and biosorption capacities of bacterial strains isolated from an urban watershed. Front. Microbiol. 14, 1278886. https://doi.org/10.3389/fmicb.2023.1278886.

Pal, C., Bengtsson-Palme, J., Rensing, C., Kristiansson, E., Larsson, D.G., 2014. BacMet: antibacterial biocide and metal resistance genes database. Nucleic Acids Res. 42 (Database issue), D737–D743. https://doi.org/10.1093/nar/gkt1252 (Jan).

Park, S., Rana, A., Sung, W., Munir, M., 2021. Competitiveness of quantitative polymerase chain reaction (qPCR) and droplet digital polymerase chain reaction (ddPCR) technologies, with a particular focus on detection of antibiotic resistance genes (ARGs). Appl. Microbiol. 1 (3), 426–444. https://doi.org/10.3390/applmicrobiol1030028.

Parsley, L.C., Consuegra, E.J., Kakirde, K.S., Land, A.M., et al., 2010. Identification of diverse antimicrobial resistance determinants carried on bacterial, plasmid, or viral metagenomes from an activated sludge microbial assemblage. Appl. Environ. Microbiol. 76 (11), 3753–3757. https://doi.org/10.1128/AEM.03080-09 (Jun).

Patel, D., Gismondi, R., Alsaffar, A., Tiquia-Arashiro, S.M., 2019. Applicability of API ZYM to capture seasonal and spatial variabilities in lake and river sediments. Environ. Technol. 40 (24), 3227–3239. https://doi.org/10.1080/09593330.2018.1468492.

Patel, H., Solanick, H., Zielinkski, E., Sweetapple, D., Tiquia-Arashiro, S., 2025. Metabolic function and carbon utilization in microbial communities of surface waters and sediments in urban river and lake watersheds. In: Watershed Microbiology: Biodiversity Composition, Ecological Function, and Technological Advances. Springer International Publishing (in press).

Pehrsson, E.C., Tsukayama, P., Patel, S., Mejía-Bautista, M., et al., 2016. Interconnected microbiomes and resistomes in low-income human habitats. Nature 533 (7602), 212–216. https://doi.org/10.1038/nature17672 (May 12).

Peltomaa, R., Benito-Peña, E., Moreno-Bondi, M.C., 2018. Bioinspired recognition elements for mycotoxin sensors. Anal. Chem. 90 (1), 124–138. https://doi.org/10.1007/s00216-017-0701-3.

Perry, J.D., Davies, A., Butterworth, L.A., et al., 2004. Development and evaluation of a chromogenic agar medium for methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. 42 (10), 4519–4523. https://doi.org/10.1128/JCM.42.10.4519-4523.2004.

Pickar-Oliver, A., Gersbach, C.A., 2019. The next generation of CRISPR-Cas technologies and applications. Nat. Rev. Mol. Cell Biol. 20 (8), 490–507. https://doi.org/10.1038/s41580-019-0131-5.

Pruden, A., Pei, R., Storteboom, H., Carlson, K.H., 2006. Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. Environ. Sci. Technol. 40 (23), 7445–7450. https://doi.org/10.1021/es060413l.

Pruden, A., Larsson, D.G.J., Amézquita, A., et al., 2013. Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. Environ. Health Perspect. 121 (8), 878–885. https://doi.org/10.1289/ehp.1206446.

Quan, J., Langelier, C., Kuchta, A., Batson, J., et al., 2019. FLASH: a next-generation CRISPR diagnostic for multiplexed detection of antimicrobial resistance sequences. Nucleic Acids Res. 47 (14), e83. https://doi.org/10.1093/nar/gkz418 (Aug 22).

Quince, C., Walker, A.W., Simpson, J.T., Loman, N.J., Segata, N., 2017. Shotgun metagenomics, from sampling to analysis. Nat. Biotechnol. 35 (9), 833–844. https://doi.org/10.1038/nbt.3935.

Rodrigues, P.A., Ferrari, R.G., Conte-Junior, C.A., 2018. Application of molecular tools to elucidate the microbiota of seafood. J. Appl. Microbiol. 124 (6), 1347–1365. https://doi.org/10.1111/jam.13701.

Rowe, W.P.M., Baker-Austin, C., Verner-Jeffreys, D.W., Ryan, J.J., Micallef, C., Maskell, D.J., Pearce, G.P., 2017. Overexpression of antibiotic resistance genes in hospital effluents over time. J. Antimicrob. Chemother. 72 (6), 1617–1623. https://doi.org/10.1093/jac/dkx017.

Said, S., Khan, S.A., 2021. Remote sensing-based water quality index estimation using data-driven approaches: a case study of the Kali River in Uttar Pradesh. India. Environ Dev Sustain 23, 18252–18277. https://doi.org/10.1007/s10668-021-01437-6.

Sanchez, A., Fenner, M., Overfield, D., Oest, A., Fortuna, A., Bakhsh, M., Zeibo, A., Tiquia-Arashiro, S., 2025. Biodegradation potential of urban watershed Bacteria for remediation across an array of organic contaminants. In: Watershed Microbiology: Biodiversity Composition, Ecological Function, and Technological Advances. Springer International Publishing (In press).

Segata, N., Waldron, L., Ballarini, A., Narasimhan, V., et al., 2012. Metagenomic microbial community profiling using unique clade-specific marker genes. Nat. Methods 9 (8), 811–814. https://doi.org/10.1038/nmeth.2066 (Jun 10).

Shin, H., Kim, Y., Han, S., Hur, H.G., 2023. Resistome study in aquatic environments. J. Microbiol. Biotechnol. 33 (3), 277–287. https://doi.org/10.4014/jmb.2210.10044 (Mar 28).

Shin, J., Kim, S.R., Xie, Z., Jin, Y.-S., Wang, Y.-C., 2024. A CRISPR/Cas12a-based system for sensitive detection of antimicrobial-resistant genes in carbapenem-resistant *Enterobacterales*. Biosensors 14 (4), 194. https://doi.org/10.3390/bios14040194.

Sommer, M.O., Church, G.M., Dantas, G., 2010. The human microbiome harbors a diverse reservoir of antibiotic resistance genes. Virulence 1 (4), 299–303. https://doi.org/10.4161/viru.1.4.12010.

Song, H.S., Kim, Y.B., Kim, J.Y., Roh, S.W., Whon, T.W., 2024. Advances in Culturomics Research on the Human Gut Microbiome: Optimizing Medium Composition and Culture Techniques for Enhanced Microbial Discovery. J. Microbiol. Biotechnol. 34 (4), 757–764. https://doi.org/10.4014/jmb.2311.11024.

Srathongneam, T., Sresung, M., Paisantham, P., et al., 2024. High throughput qPCR unveils shared antibiotic resistance genes in tropical wastewater and river water. Sci. Total Environ. 908, 167867. https://doi.org/10.1016/j.scitotenv.2023.167867.

Stedtfeld, R.D., Guo, X., Stedtfeld, T.M., Sheng, H., et al., 2018. Primer set 2.0 for highly parallel qPCR array targeting antibiotic resistance genes and mobile genetic elements. FEMS Microbiol. Ecol. 94 (9), fiy130. https://doi.org/10.1093/femsec/fiy130 (Sep 1).

Sudriani, Y., Sebestyén, V., Abonyi, J., 2023. Surface water monitoring systems—the importance of integrating information sources for sustainable watershed management. IEEE Access 11, 36421–36451. https://doi.org/10.1109/ACCESS.2023.3263802.

Sun, Y., Clarke, B., Clarke, J., Li, X., 2021. Predicting antibiotic resistance gene abundance in activated sludge using shotgun metagenomics and machine learning. Water Res. (202), 117384. https://doi.org/10.1016/j.watres.2021.117384 (Sep 1).

Sunagawa, S., Coelho, L.P., Chaffron, S., et al., 2015. Structure and function of the global ocean microbiome. Science 348 (6237), 1261359. https://doi.org/10.1126/science.1261359.

Tao, S., Chen, H., Li, N., Liang, W., 2022. The application of the CRISPR-Cas system in antibiotic resistance. Infect Drug Resist. (15), 4155–4168. https://doi.org/10.2147/IDR.S370869 (Aug 2).

Tiquia, S.M., 2010. Salt-adapted bacteria isolated from the Rouge River and potential for degradation of contaminants and biotechnological applications. Environ. Technol. 31, 967–978. https://doi.org/10.1080/09593331003706226.

Tiquia-Arashiro, S.M., 2012. Molecular biological technologies for ocean sensing. In: Springer Protocols Handbooks. Humana, Totowa, NJ. https://doi.org/10.1007/978-1-61779-915-0 (307 p.).

Tiquia-Arashiro, S.M., 2018. Lead absorption mechanisms in bacteria as strategies for lead bioremediation. Appl. Microbiol. Biotechnol. 102 (13), 5437–5444. https://doi.org/10.1007/s00253-018-8969-6.

Tiquia-Arashiro, S.M., Pant, D., 2020. Microbial Electrochemical Technologies. CRC Press, Boca Raton, Florida, USA, p. 518. https://doi.org/10.1201/9780429487118.

Tiquia-Arashiro, S.M., Medema, G., Ubomba-Jaswa, E., Urakawa, H., Ella, V., Libardi, N., 2025. Watershed Microbiology: Biodiversity Composition, Ecological Function, and Technological Advances. Springer International Publishing (In press).

Turner, A.P.F., 2013. Biosensors: sense and sensibility. Chem. Soc. Rev. 42 (8), 3184–3196. https://doi.org/10.1039/C3CS35528D.

Van Boeckel, T.P., Brower, C., Gilbert, M., et al., 2015. Global trends in antimicrobial use in food animals. Proc. Natl. Acad. Sci. USA 112 (18), 5649–5654. https://doi.org/10.1073/pnas.1503141112 (May 5).

Wang, D.N., Liu, L., Qiu, Z.G., Shen, Z.Q., et al., 2016. A new adsorption-elution technique for the concentration of aquatic extracellular antibiotic resistance genes from large volumes of water. Water Res. (92), 188–198. https://doi.org/10.1016/j.watres.2016.01.035 (Apr 1).

Wang, X., Gu, J., Gao, H., Qian, X., Li, H., 2018. Abundances of clinically relevant antibiotic resistance genes and bacterial community diversity in the Weihe River, China. Int. J. Environ. Res. Public Health 708. https://doi.org/10.3390/ijerph15040708.

Wang, B., Wang, H., Lu, X., Zheng, X., Yang, Z., 2023. Recent advances in electrochemical biosensors for the detection of foodborne pathogens: current perspective and challenges. Foods 12 (14), 2795. https://doi.org/10.3390/foods12142795.

Wang, C., Mao, Y., Zhang, L., Wei, H., Wang, Z., 2024. Insight into environmental adaptability of antibiotic resistome from surface water to deep sediments in anthropogenic lakes by metagenomics. Water Res. (256), 121583. https://doi.org/10.1016/j.watres.2024.121583 (Jun 1).

Wattam, A.R., Davis, J.J., Assaf, R., Boisvert, S., Brettin, T., et al., 2017. Improvements to PATRIC, the all-bacterial Bioinformatics Database and Analysis Resource Center.

Nucleic Acids Res. 45 (D1), D535–D542. https://doi.org/10.1093/nar/gkw1017 (Jan 4).

Wen, B., Mei, Z., Zeng, C., et al., 2017. metaX: a flexible and comprehensive software for processing metabolomics data. BMC Bioinformatics 18, 183. https://doi.org/10.1186/s12859-017-1579-y.

Xie, Z., Zhao, Y., Jiang, R., Zhang, M., et al., 2024. Seasonal dynamics of fallow and cropping lands in the broadacre cropping region of Australia. Remote Sens. Environ. 305, 114070. https://doi.org/10.1016/j.rse.2024.114070.

Xiong, W., Sun, Y., Zhang, T., et al., 2015. Antibiotics, antibiotic resistance genes, and bacterial community composition in fresh water aquaculture environment in China. Microb. Ecol. 70 (2), 425–432. https://doi.org/10.1007/s00248-015-0583-x (Aug).

Xu, N., Qiu, D., Zhang, Z., Wang, Y., et al., 2023. A global atlas of marine antibiotic resistance genes and their expression. Water Res. (244), 120488. https://doi.org/10.1016/j.watres.2023.120488 (Oct 1).

Yang, Y., Li, B., Ju, F., Zhang, T., 2013. Exploring variation of antibiotic resistance genes in activated sludge over a four-year period through a metagenomic approach. Environ. Sci. Technol. 47 (18), 10197–10205. https://doi.org/10.1021/es4017365 (Sep 17).

Zhang, X.X., Zhang, T., Fang, H.H., 2009. Antibiotic resistance genes in water environment. Appl. Microbiol. Biotechnol. 82 (3), 397–414. https://doi.org/10.1007/s00253-008-1829-z (Mar).

Zhang, Y., Li, J., Jiao, S., et al., 2024. Microfluidic sensors for the detection of emerging contaminants in water. Sci. Total Environ. (929), 172734. https://doi.org/10.1016/j.scitotenv.2024.172734 (Jun 15).

Zhao, J., Yan, Y., Zhu, L., Li, X., Li, G., 2013. An amperometric biosensor for the detection of hydrogen peroxide released from human breast cancer cells. Biosens. Bioelectron. 41, 815–819. https://doi.org/10.1016/j.bios.2012.10.019.

Zhao, Y., Huang, F., Wang, W., Gao, R., Fan, L., Wang, A., Gao, S.H., 2023. Application of high-throughput sequencing technologies and analytical tools for pathogen detection in urban water systems: progress and future perspectives. Sci. Total Environ. (900), 165867. https://doi.org/10.1016/j.scitotenv.2023.165867 (Nov 20).

Zheng, Z., Zhang, Y., Liu, Z., Dong, Z., Xie, C., Bravo, A., Soberón, M., Mahillon, J., Sun, M., Peng, D., 2020. The CRISPR-Cas systems were selectively inactivated during evolution of Bacillus cereus group for adaptation to diverse environments. ISME J. 14 (6), 1479–1493. https://doi.org/10.1038/s41396-020-0623-5.

Zhu, X., 2016. GIS for Environmental Applications: A Practical Approach, 1st ed. Routledge, London. https://doi.org/10.4324/9780203383124. (490 p.).

Zhu, Y.G., Johnson, T.A., Su, J.Q., et al., 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proc. Natl. Acad. Sci. 110 (9), 3435–3440. https://doi.org/10.1073/pnas.1222743110.

Zhu, Y.G., Zhao, Y., Li, B., Huang, C.L., et al., 2017. Continental-scale pollution of estuaries with antibiotic resistance genes. Nat. Microbiol. (2), 16270. https://doi.org/10.1038/nmicrobiol.2016.270 (Jan 30).