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Editorial

Monitoring Antimicrobial Resistance Dissemination in Aquatic Systems

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Abstract: This special issue on Antimicrobial Resistance in Environmental Waters features 11 articles on monitoring and surveillance of antimicrobial resistance (AMR) in natural aquatic systems (reservoirs, rivers), and effluent discharge from water treatment plants to assess the effectiveness of AMR removal and resulting loads in treated waters. The occurrence and distribution of antimicrobials, antibiotic resistant bacteria (ARB), antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) was determined by utilizing a variety of techniques including liquid chromatography—mass spectrometry in tandem (LC-MS/MS), traditional culturing, antibiotic susceptibility testing (AST), molecular and OMIC approaches. Some of the key elements of AMR studies presented in this special issue highlight the underlying drivers of AMR contamination in the environment and evaluation of the hazard imposed on aquatic organisms in receiving environments through ecological risk assessments. As described in this issue, screening antimicrobial peptide (AMP) libraries for biofilm disruption and antimicrobial candidates are promising avenues for the development of new treatment options to eradicate resistance. This editorial puts into perspective the current AMR problem in the environment and potential new methods which could be applied to surveillance and monitoring efforts.

Keywords: Antimicrobial Resistance; Environmental Waters; water treatment plants; water reuse; ecological risk assessment

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1. Introduction

The release of antimicrobials, antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) originating from human and animal waste to the environment is a global problem which has serious ramifications on public health. In response to this growing health threat, the World Health Organization (WHO) launched a global action plan on Antimicrobial Resistance (AMR) in 2015 with 5 strategic objectives, one of which was to strengthen knowledge of the spread of AMR through surveillance and research [1]. As a guide, the WHO has drawn up a priority list of AMR pathogens based on the threat they pose on human infections, response to antibiotic treatment, transmissibility between humans and animals, and whether there are antibiotics in current research and development pipelines to treat infections caused by these pathogens. Those of highest priority are carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant *Pseudomonas aeruginosa*, and carbapenem-resistant, extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* [2]. The WHO's Global Antimicrobial Surveillance System (GLASS) report for 2018 revealed widespread occurrence of antibiotic resistance among half a million people with suspected bacterial infections across 22 countries [3]. An AMR risk assessment of the South East Asian Region by Chereau et al. [4] concluded a high likelihood of emergence and dissemination among humans. Low stewardship on antibiotic prescription in treatment of human infections and the absence of legal frameworks for antibiotic use in animal husbandry and aquaculture are the main drivers for the selection of ARB in South East Asia [5]. The direct release or insufficient treatment of wastewater effluents from healthcare, livestock, aquaculture, and agriculture sites into receiving environments also poses a significant risk. In 2013, China alone produced 92,700 tonnes of antibiotics, 48% of which were consumed by humans and 52% by animals [6]. It was reported that almost half of all antibiotics were released in rivers through wastewater effluents and the practice of manure and sludge land spreading [6].

In South East Asia, AMR risk ranking across humans, animals, and environmental compartments show that human to human transmission in community and hospital settings represent the highest risk of the emergence and selection of AMR vectors (ARB, ARGs), followed by food- and waterborne transmission to humans through ingestion of contaminated sources [4]. Although transmission via contact with contaminated environments (through soil, water, and air), and livestock/animals is regarded as low risk in comparison to human to human transmission, it is still considered a valid route of exposure, particularly in countries that are water-scarce and reliant on water reuse. Hence, it is crucial to address the impact of antimicrobials, ARB and ARGs on AMR prevalence in receiving environments, specifically in countries where data availability is scant and guidelines for AMR stewardship frameworks have yet to be established. It is only through identifying and tracking sources and sinks of AMR in the environment, where intervention strategies can be devised to prevent and control the spread of the problem.

2. Measuring Vectors of AMR

2.1. Antimicrobials

Currently, there are various practices and methods of monitoring AMR dissemination and the fate of ARB and ARGs in aquatic environmental systems. Highly sensitive analytical protocols have been developed to detect antimicrobials using liquid chromatography-tandem mass spectrometry in environmental water samples and effluents from wastewater treatment plants (WWTPs) [7,8], while culture-based techniques are used to enumerate ARB.

2.2. ARB

Most environmental and wastewater treatment AMR surveys use methods applied in clinical settings, where media is supplemented with antibiotics at concentrations above the recommended minimum inhibitory concentration (MIC) breakpoints implemented by the Clinical and Laboratory Standards Institute [9] or the European Committee on antimicrobial Susceptibility Testing [10–17]. For non-clinically relevant bacteria (such as environmental bacteria), epidemiological cut-off values (ECOFF) are an alternative method used to gauge non-wild-type bacteria that display reduced susceptibility to certain antimicrobials or biocides [18]. Further testing of multidrug resistance (MDR) of ARB isolates are performed using broth dilution assays, Kirby-Bauer disk diffusion test on Muller-Hinton agar, or high-throughput platforms such as the VITEK system by bioMerieux (France).

2.3. ARGs, MGEs

To detect the prevalence of ARGs and vectors such as mobile genetic elements (MGE) that may facilitate horizontal gene transfer of ARGs, traditional quantitative polymerase chain reaction (qPCR), and the more recent high-throughput qPCR (HT-qPCR) platform with capabilities of detection of ~200 different ARGs and mobile genetic elements (MGE), has been used to compare relative concentrations of AMR contamination across a variety of aquatic environments including water treatment plants [19–24]. OMIC approaches such as metagenomics are able to provide a holistic picture of the diversity of ARGs, MGE and vectors (e.g., integrons, plasmids) that assist horizontal gene transfer, and the overall microbial community structure (bacteria, viruses) in environmental systems and wastewaters [25–29]. ResCap, a targeted capture platform (TCP) designed to analyze ~78,000 ARGs, metal resistance and plasmid markers is a targeted metagenomics approach for qualitative and quantitative resistome analysis [30]. Other OMIC approaches, such as metatranscriptomics, enable the identification of active microbial members within a community and, in the context of AMR, enables the measurement of transcription activity of ARB through ARG expression [31].

3. Key Outcomes of this Special Issue

This special issue comprises of 11 research articles that fall within the scope of AMR. Broadly, topics extend from AMR monitoring and surveillance in environmental resources and effluents from water/wastewater treatment plants [32–39], antimicrobial ecological risk assessments of two river reservoir systems in China that are sources of drinking water supply [40,41], and exploring novel strategies of using engineered antimicrobial peptides (AMP) to target specific bacteria to disrupt biofilms that are major causes of chronic and persistent infections [42].

AMR Monitoring and Surveillance

Knowledge of removal efficiencies of technology employed at WWTPs is essential in AMR surveillance, whether effluents are intended for discharge into the environment or reuse for irrigation purposes.

To determine the presence of antibiotic resistant *E. coli* in a conventional WWTP in Georgia, Aslan et al. [36] isolated *E. coli* from post-secondary, post-UV and post-chlorination effluent and performed antibiotic susceptibility tests on the isolates. They reported that ~5.2 log removal of *E. coli* and an additional 1.1 log reduction post chlorination was obtained. However, the MICs of *E. coli* isolated in the finished water were higher than those at the other treatment stages. The selection of more resistant organisms in the finished water underscores the urgent need to evaluate the health risks of using reclaimed water for downstream irrigation. In a qPCR assessment of ARGs of a full-scale tertiary water reclamation plant, Quach-Cu et al. [39] showed that tertiary-stage WWTPs with disinfection had superior removal of ARGs (*sul1*, $bla_{\mathrm{SHV/TEM}}$), ~3–4 logs compared to reliance of secondary treatment alone where the removal was only ~1–3 logs. To assess the impact of treated effluent on receiving environments, Lambirth et al. [35] measured the removal efficiency of ten antibiotics and assayed resistomes upstream, downstream and within various treatment steps of two urban WWTPs (secondary and disinfection treatments). The authors found elevated concentration of all 10 antibiotics surveyed in downstream receiving waters compared to waters upstream of the WWTP. The relative abundance of ARG signatures encoding for resistance to carbapenems and ESBL antibiotics were much lower than those detected upstream and sampling points within the WWTP, which debunks the notion that the wastewater treatment process selects for ARG resistance. Instead, the authors hypothesized that antibiotics discharged from treated wastewater effluent into the downstream environment may have an effect on natural microbial communities.

Jumat et al. [37] conducted an ESBL study of diversity and transcriptional activity of bacteria in a WWTP in Saudi Arabia using metagenomics, metatranscriptomics and real-time qPCR. They found an increase in the relative abundance of *Acinetobacter junii* in MBR- and chlorinated treated effluent. Survival and predominance of *A. junii* was explained by metatranscriptomics data that showed an upregulation of gene associated with active cell repair, resistance, virulence (efflux transporters involved in metal and antibiotic resistance) and cell signaling. These adaptive cellular mechanisms enable *A. junii* to withstand depletion of nutrients and counter the effects of chlorination. However, the authors indicate that the low concentrations of viable *A. junii* isolated from MBR effluents may not present that huge a risk. The varying results from WWTP studies covered in this issue makes it challenging to establish whether WWTPs are indeed hotspots of AMR dissemination. Rather, it is likely that differences in global and plant operating process contribute to variation in antibiotic resistance elements detection.

In countries with high agricultural productivity, water reuse is commonly practiced to meet the high water demands of the industry [43]. On a global scale, there are no clear guidelines implemented on assessment of water quality for reuse purposes, although a few countries have drawn up recommended microbiological parameters to monitor the quality of recycled water [44]. A recent review by Hong et al. [44] highlights the urgency of understanding the risks of microbiological and ARG contamination linked to water reuse. In the Philippines, surface waters contaminated with fecal coliform are frequently used for irrigating urban farms in densely populated cities [45]. Vital et al. [33] evaluated the antibiotic resistance profiles of 212 *E. coli* strains isolated from irrigation water, soil, and vegetables from six urban agricultural farms. Of the total isolates, 36.5% were resistant to more than three antibiotics tested, with the most multidrug-resistant (MDR) isolates being detected in irrigation water, followed by soil and vegetables. Of the MDR *E. coli* isolated from irrigation water, 7 of them were ESBL producers that carried either bla_{TEM} or $bla_{\mathrm{CTX\text{-}M}}$ genes, which raises public health concerns in primary production environments such as agricultural soils and fresh produce grown in these areas.

The use of antimicrobials in food animals is widespread, and runoffs originating from animal waste may carry unmetabolized antibiotics or ARB and ARGs that have direct impact on surrounding water bodies [46]. Tsai et al. [32] linked significantly higher concentrations of *A. baumannii* along the Puzi River in China to sampling sites of livestock wastewater channels and tributaries adjacent to livestock farms. Further testing of 20 *A. baumannii* isolates against 7 antibiotics (ciprofloxacin, cefepime, gentamicin, imipenem, ampicillin-sulbactam, sulfamethoxazole/trimethoprim, tetracycline) by the Kirby-Bauer disk diffusion test showed that only 10% had resistance to sulfamethoxazole/trimethoprim and 5% had resistance to tetracycline. Although the *A. baumannii* isolated by Tsai et al. [32] did not display MDR patterns which are regarded a serious AMR threat by WHO, their epidemiological potential warrants further studies on prevalence and AMR developmental trends in the environment.

In China, the Yellow River in the North serves as an important source of drinking water and is flanked by cities along its banks and watersheds. Previous studies have reported high turbidity and concentrations of antimicrobials in the Yellow River Catchment [47]. This prompted Lu et al. [38] to investigate the distribution and abundance of ARGs in Sand Settling Reservoirs (SSR) and Drinking Water Treatment Plants (DWTP) along the Yellow River. By targeting 17 ARGs as a proxy for AMR removal through the treatment process, the total concentrations of ARGs decreased from 10^4 copies/mL in influent river waters to 10^3 copies/mL in SSR effluent to 10^2 copies/mL in finished water. The 2 MGE targets decreased by at least an order of magnitude, from 10^6 copies/mL in influent river waters to 10^5 copies/mL in finished waters.

Horizontal gene transfer (HGT) of ARGs in the mammalian gut through the ingestion of contaminated food or water generally poses a low risk due to harsh conditions in the gut. However, this may present a greater risk for propagation in the environment through transformation and transduction [44]. In another study by Xu et al. [34], the authors studied the relationship of 16 antibiotics with environmental water quality parameters and the impact of antibiotic concentrations on the microbial community structure along Qingcaosha Reservoir, the largest estuary reservoir in China. This reservoir has a similar function to the Yellow River, in that it compensates for drinking water shortages in Shanghai. From the study, the authors concluded that upstream runoffs and anthropogenic activity along the river contributed to the concentrations of antibiotics measured within the reservoir, and that tylosin, penicillin G and erythromycin-H_2O showed significant correlations with variations in bacterial community structure. Further to this study, Jiang et al. [40] studied seasonal variations of antibiotics in surface waters of Qingcaosha Reservoir. By using risk quotients (RQs) based on the European technical guidance documentation (TGD) on risk assessment, they showed that out of the 17 antibiotics monitored, four antibiotics (doxycycline, penicillinV, norfloxacin, ciprofloxacin) posed a high risk to relevant sensitive aquatic organisms, as well as imposed selective stress on microbial communities. In another study, of a subtropical river-reservoir system located in the Headwater Region of the Dongjiang River which supplies drinking water to three major cities in China, Chen et al. [41] conducted an ecological risk assessment which showed that concentrations of ciprofloxacin and norfloxacin posed a moderate risk, while tetracycline posed a higher risk to the aquatic ecosystem.

Finally, through screening a local antimicrobial peptide (AMP) library, Chin et al. [42] identified LG21, an AMP that specifically binds to exopolysaccharide PsI of *P. aeruginosa* that has a functional role of biofilm formation, which provides a protective environment for tolerance and resistance towards antibiotic treatment. This strategy of exploring AMP to target specific biofilm matrix components to disrupt formation and development of biofilms is a promising line of treatment to eradicate antibiotic resistant biofilms in both environmental and clinical settings.

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