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Review Article

Detection of antimicrobial resistance genes (ARGs) in surface waters and the anthropogenic factors influencing its abundance: A systematic review

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Abstract
Antimicrobial resistance (AMR) is a global public health threat, and several studies have aimed to identify resistant pathogens and/or associated antimicrobial resistance genes in surface waters. A systematic review of 98 studies was done to obtain a bigger picture of detecting antibiotic resistance genes (ARGs) across surface waters and the factors influencing its abundance. Available data revealed a high abundance and detection rate of ARGs conferring resistance to critically important antibiotics. Tetracycline and sulfonamide resistance genes were the most frequently studied within the past 10 years. The highest reported abundance of ARGs is up to 10^{11} copies per mL for *ermF* gene, quantified using quantitative PCR (qPCR). More advanced methods such as metagenomic sequencing, digital droplet PCR (ddPCR), and high-throughput quantitative PCR (HT-qPCR) identified greater numbers of ARGs per run compared to the conventional PCR and qPCR methods. This study emphasized the role of surface waters in the dissemination of ARGs. Surface waters not only harbor ARGs but are also significantly influenced by human activities, which alter ARG concentrations, demonstrating a two-way relationship between human activities and the environment, where surface waters serve both as recipients and conduits of ARGs, reflecting the complex interplay between anthropogenic influences and environmental systems. Recommendations for future directions in AMR surveillance studies are also provided.

Keywords: Antibiotic resistance genes (ARG) abundance, Antimicrobial resistance genes, Assays, Surface water

INTRODUCTION

Antimicrobial agents have been useful to society; however, the continuous overuse and mismanagement of antibiotics have led to resistant strains. Moreover, medium-income countries are found to be more prone to antibiotic misuse, which further contributes to the rise of resistant strains (Mallah *et al.,* 2022). According to the World Health Organization, (WHO), antimicrobial resistance (AMR) is one of the top global threats in fighting and controlling infectious diseases (WHO 2024). The continued increase in the incidence of AMR has led to significant delays in the timely administration of appropriate antibiotic treatments, consequently contributing to higher rates of illness and death (Bassetti *et al.,* 2021). Global studies have revealed data gaps regarding AMR in environmental microorganisms, and such knowledge gaps need to be investigated to better understand the role of environmental factors in driving evolutionary processes that contribute to resistance as well as aid in minimizing the impact of environmental exposure to antibiotic residues on humans and reducing the related health risks (Ahmad *et al.,* 2021).
Antibiotic-resistant bacteria (ARB) have been detected in aquatic environments, including irrigation ponds, rural rivers, and watersheds (Yang *et al.,* 2020; Peng *et*

al., 2020; Shamsizadeh *et al.,* 2021). The widespread incidence of ARB in surface waters indicates that it may play a role in transmitting antibiotic resistance determinants. Human activities, such as recreational and economic activities constantly influence surface waters. Wastewater draining into water channels is also a key source of ARB (Cho *et al.,* 2020).

On the other hand, natural phenomenon like rainfall also contributes to the dissemination of ARGs from surrounding communities to nearby natural waters (Wang *et al.,* 2021; Williams *et al.,* 2022). Various recreational and economic activities involve surface water, potentially exposing people to antibiotic-resistant pathogens. Therefore, monitoring surface water quality and the presence of ARB is of public health importance.

AMR is a global public health threat, and several studies have aimed to identify AR pathogens and/or associated antimicrobial resistance genes in surface waters. However, the overall picture of AMR in surface waters cannot be seen due to various limitations in the previous studies, such as the difference in terms of the species studied, the methodology used, and the type of surface water investigated. Without understanding the status of AMR in surface waters, knowledge gaps still exist, compromising the ability to implement or craft policies that can truly address the issue of AMR. With this systematic review, the present study aimed to find out a bigger picture of the current state of AMR in surface waters to identify current gaps and propose future directions for AMR research.

METHODOLOGY

This systematic review was based on Moher's adapted version of the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) statement (2019). Three databases (Google Scholar, Scopus, and Web of Sciences) were searched using the following keywords: “antimicrobial resistance gene*” OR “antimicrobial resistance determinant” AND “surface water” OR stream* OR river* OR lake* OR reservoir* OR ocean* OR creek* OR pond* OR “environmental water.” The search approach for initial pooling of research papers was designed by authors. Records obtained from the three databases were merged into one spreadsheet. From this point onwards, Microsoft Excel was used to organize the records.

Following the workflow as illustrated in Fig.1, a total of 297 studies remained and the following inclusion criteria were used to select the studies that were used in systematic review: i) Studies which assessed the abundance/level/concentration/frequency and/or presence/absence of ARGs in surface water, ii) Studies must include data on related human activity near or around the surface water; iii) Studies with cross-sectional design.

Finally, 98 research articles were selected. In this study, it was also attempted to perform a meta-analysis using the following criteria:i) Used quantitative polymerase chain reaction (qPCR) as the method of detection and quantification; ii) Used copies/mL as the quantification unit; and iii) Complete raw data per sampling point is available. However, only nine studies passed the above-mentioned eligibility criteria and processed for meta-analysis.

Based on the eligibility criteria, 98 studies on the detection of ARGs in surface water were included in the systematic review. These studies consisted of 55 studies which used qPCR, 15 which used conventional PCR, 11 studies which used metagenomic/whole genome

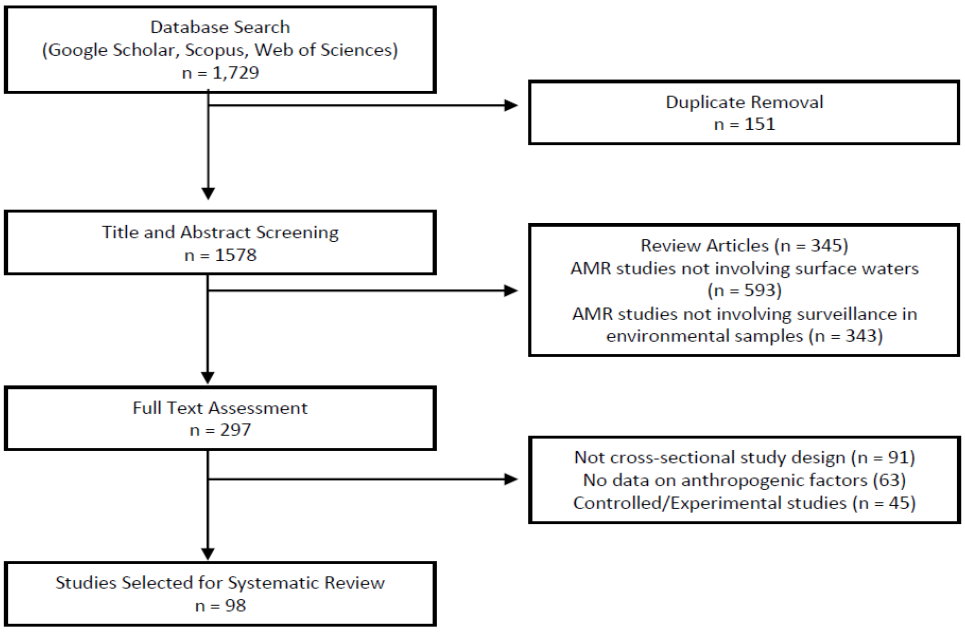

Fig. 1. *Systematic review process used in this study*

sequencing, 2 studies which used digital droplet PCR (ddPCR), 9 studies which used high-throughput qPCR (HT-qPCR), and 6 studies which used a combination of the said methods on the detection and quantification of ARGs. Further screening based on eligibility criteria selected 9 studies on determining the abundance of ARGs in surface water using the qPCR. Articles searched are within the last 10 years as of February 2023, when the database search was conducted. For the systematic review, the studies were from 31 countries namely, China, India, Poland, India, Japan, Germany, Spain, Brazil, France, Nepal, Puerto Rico, Romania, Australia, Austria, Benin, Bolivia, Bulgaria, Croatia, Czech Republic, Ethiopia, Hungary, Iran, Ireland, Nigeria, Serbia, South Korea, Sri Lanka, Sri Lanka, Thailand, United Kingdom, Uruguay, and USA (California, Georgia, and Nebraska). Of the 98 studies included, 53 were studies from China. Various types of surface waters were investigated in the selected studies, but the majority of which were focused on ARGs in rivers (Fig. 2). Of note, since it was observed in the review that the authors of the studies included mostly did not state their specific rationale behind which ARGs they are going to detect and quantify in the studies, from this point onward, the term “targeted” will be used instead of “detected” whereby appropriate to emphasize that authors in the selected studies pre-defined their ARGs of interest. Therefore, the low occurrence of some ARG types or subtypes, as it may appear in this review paper, does not necessarily equate to their being absent or low in the environment.

RESULTS AND DISCUSSION

Frequency and concentrations of selected ARGs detected through conventional methods and qPCR

Fifteen studies met the eligibility criteria for conventional PCR. The following ARG class were found most frequently: aminoglycoside (32.16%), beta-lactamase (38.67%), chloramphenicol (46.51%), diaminopyrimidine (26.96%), macrolide (47.98%), quinolone (12.86%), sulfonamide (50.88%), tetracycline (28.98%), vancomycin (59.07%) (Table 1). The detection rate of vancomycin was found to be high, which is quite alarming. Globally, surface waters, wastewater treatment plants, home sewage, and hospital sewage all have vancomycin resistance genes. The likelihood of transmission to other Gram-positive pathogens may rise because of the environmental distribution of genes conferring vancomycin resistance (Young *et al.,* 2016). Vancomycin-resistant enterococci (VRE) are becoming more prevalent and constitute a significant treatment challenge. The human gastrointestinal tract, animals, the medical setting, and wastewater are just a few examples of the various potential sources of VRE. Antimicrobial residues may, among other things, be connected to the prevalence of VRE in the environment (Hricová *et al.,* 2021). On the other hand, it was found that quinolones had the lowest detection rate among the ARG classes.

The abundance of selected ARGs across various surfaces detected via qPCR are shown in (Table 2a and Table 2b). Among the qPCR studies reviewed, the *ermF*, which confers resistance to macrolides, was the ARG that was detected with the highest concentration among the studies reviewed at 1.00E+11 copies/mL (Ahmed *et al.,* 2021). Meanwhile, *tetM*, a gene conferring resistance to tetracyclines, and sul2, a gene conferring resistance to sulfonamides, were the most frequently studied ARGs and were also detected at high levels. The macrolide, tetracycline, and sulfonamide antibiotics are used extensively and continuously in veterinary and human medicine and are frequently discovered in various aqueous matrices (Pérez *et al.,* 2017; Zhang *et al.,* 2022; Paulus *et al.,* 2020). Tetracycline resistance genes (TRGs) and tetracycline-

Fig. 2. *Overview of studies selected for systematic review*

Table 1. Antibiotic resistance genes (ARGs) across various surface waters detected through conventional polymerase chain reaction (PCR)

ARG Class	Detected Positive	Total Number of Samples	Detection Rate	References
Vancomycin Resistance Genes	244	413	59.08	Nishiyama *et al.* 2017; Ichola *et al.* 2021; Yang *et al.* 2018
Sulfonamide Resistance Genes	754	1482	50.88	Chaturvedi *et al.* 2021; Kumar *et al.,* 2020; Lazar et al, 2021; Mala *et al.* 2017; Sala-Comorera *et al.* 2021; Ravi *et al,* 2022; Zhang *et al.* 2015; Yang *et al.* 2018; Titilawo *et al.* 2015; Jiang *et al.* 2018b; Yang *et al.* 2017
Macrolide Resistance Genes	120	245	48.98	Yang *et al.* 2018; Leclercq *et al.* 2013; Jiang *et al.* 2018b; Silva *et al.* 2021
Chloramphenicol Resistance Genes	80	172	46.51	Ravi *et al,* 2022; Titilawo *et al.* 2015; Jiang *et al.* 2018b; Silva *et al.* 2021
Beta-lactamase Resistance Genes	817	2113	38.67	Chaturvedi *et al.* 2021; Kumar *et al.,* 2020; Lazar et al, 2021; Kittinger *et al.* 2018; Dhawde, *et al.,* 2018; Ichola *et al.* 2021; Sala-Comorera *et al.* 2021; Ravi *et al,* 2022; Zhang *et al.* 2015; Yang *et al.* 2018; Lenart-Boron 2017; Jiang *et al.* 2018b; Shahin *et. al*. 2019; Silva et. al. 2021
Aminoglycoside Resistance Genes	401	1247	32.16	Kumar et al, 2020; Lazar *et al,* 2021; Zhang *et al*, 2015; Yang *et al.* 2018; Titilawo *et al,* 2015; Shahin *et al.* 2019; Silva *et al.* 2021; Mala *et al.* 2017
Tetracycline Resistance Genes	364	1256	28.98	Lazar *et al.,* 2021; Mala *et. al.* 2017; Ravi *et al.,* 2022; Zhang *et al.* 2015; Yang *et al.* 2018; Leclercq *et al.* 2013; Titilawo *et al.* 2015; Jiang *et al.* 2018b; Shahin et. al. 2019; Silva *et al.* 2021; Yang *et al.* 2017
Diaminopyrimidine Resistance Genes	93	345	26.96	Chaturvedi et al. 2021; Mala *et. al.* 2017; Silva *et al.* 2021
Quinolone Resistance Genes	76	591	12.86	Kumar *et al.,* 2020; Dhawde, *et. al* 2018; Ichola *et al.* 2021; Sala-Comorera *et al.* 2021; Ravi *et al,* 2022; Zhang *et al.* 2015;Yang *et al.* 2018

resistant bacteria (TRBs) have become increasingly common in aquaculture environments in recent years because of the frequent detection of TC in ecosystems of various watersheds, including the Yellow Sea, East Dongting Lake, and Taihu Lake in China (Zhang *et al.,* 2022). For almost as long as they have been used to treat infectious diseases, tetracycline antibiotics have also been utilized to encourage development in systems that produce animals for food (Adesoji *et al.,* 2015). Due to their low cost, sulfonamides, a type of synthetic veterinary antibiotic, are the most often used antibiotics in China, the European Union, and certain underdeveloped nations. It has been suggested that ARGs and mobile genetic elements (MGEs), like sulfonamide resistance genes can be used as proxies to track anthropogenically generated ARGs in the environment. Sulfonamide resistance genes, such as *sul1*, have been suggested to indicate ARG contamination in agricultural pollution and urban places (Paulus *et al.,* 2020). Sulfonamides, furthermore, were ranked as "high priority" veterinary medications because of their considerable potential for environmental exposure. Sulfonamides are very soluble in water and have good chemical stability making them easily found in surface water.

It has been discovered that natural water contains many sulfonamides, which could raise the total risk due to the combination effect (Qin *et al.,* 2020). These ARGs are regarded as emerging pollutants that may exist in surface water and pose concerns to human

Table 2a. Abundance of major antibiotic resistance genes (ARGs) across various surface waters detected through qPCR (copies/mL)

Antibiotic Class	Resistance Genes	Concentration (copies/mL)	Reference
Beta-lactam	*blaTEM*	2.90 × 10 to 6.00 × 10^6	Liyanage *et al.* 2021; Jang *et al.* 2022; Stange *et al.* 2018; Harnisz et al. 2020
Macrolide	*ermA*	2.41 × 10^8	Yang *et al.* 2019
	ermB	1.15 × 10 to 1.65 × 10^5	Reichert *et al.* 2021; Zhang *et al.* 2020a; Zhao *et al.* 2021
	ermF	1.00 × 10^{11}	Ahmed *et al.* 2021
Colistin	*MCR-1*	3.30 × 10^9	Wang *et al.* 2019
	NDM-1	9.50 × 10^7	Wang *et al.* 2019
Quinolone	*oqx(B)*	2.29 × 10^{-3} to 3.49 × 10^{-2}	Xiong *et al.* 2014
	qnrB	2.51 × 10^{-2} to 4.91 × 10^{-3}	Han et al. 2013
	qnrS	1.51 × 10^{-4} to 3.92 × 10^6	Han *et al.* 2013; Zhang *et al.* 2020a; Zhao *et al.* 2021
Aminoglycoside	*strA*	6.33 × 10^{-6} to 1.12 × 10^2	Lu *et al.* 2018; Han *et al.* 2013
	strB	1.25 × 10^{-4} to 1.07 × 10^4	Lu *et al.* 2018; Han *et al.* 2013
Sulfonamide	*sul1*	1.00 × 10^1 to 3.16 × 10^9	Xiong *et al.* 2014; Reichert *et al.* 2021; He *et al.* 2022; Hubeny *et al.* 2021; Chen *et al.* 2020a; Stange *et al.* 2018; Ahmed *et al*. 2021; Zhang *et al.* 2020a; Zhao *et al.* 2021; Harnisz *et al.* 2020; Li *et al.* 2020; Makowska *et al.* 2016; Truong *et al.* 2021; Li and Zhang 2020
	sul2	2.76 × 10^1 to 1.16 × 10^{10}	Zou *et al.* 2021; Hubeny *et al.* 2021; Xu *et al.* 2019; Zhang *et al.* 2020a; Zhao et *al.* 2021; Makowska *et al.* 2016; Truong *et al.* 2021
Tetracycline	*tetW*	1.22 × 10^{-3} to 8.93 × 10^2	Xiong et al. 2014; Zhao *et al.* 2021
	tetX	1.19 × 10^6	Chen *et al.* 2020a
	tetA	4.40 × 10^4	Liyanage *et al.* 2021
	tetM	2.72 × 10^{10}	Xu *et al.* 2019
Chloramphenicol	*floR*	7.84 × 10^5	Li *et al*. 2020
	cmlA	2.04 × 10^5	Li *et al.* 2020
Trimethoprim	*dfrA*	2.7 × 10^2 to 0.5 × 10^7	Truong *et al.* 2021
Integrons	*Int1*	6.56 × 10^2 to 1.45 × 10^7	Zou *et al.* 2021; Chen *et al.* 2020; Harnisz *et al.* 2020; Koczura *et al*. 2016
	Int2	1.00 × 10^6	Yang *et al.* 2019

health and the environment. Antibiotic resistance has an impact on the long-term sustainability of health, food security, clean water, and sanitation. It also seriously risks human health and the economy (Zhang *et al.,* 2022).

Using the data from 9 qPCR studies which passed the inclusion criteria, a meta-analysis of the abundance of selected ARGs in surface water was attempted. However, the test for heterogeneity (I2) showed a significant variance between the studies. Hence, the pooled mean abundance of ARGs cannot be obtained (Table S1). A variety of factors observed in the studies can corroborate this finding. First and most importantly are the differences in the critical aspects of the research design of the reviewed studies, such as the number of subjects (surface water investigated), number of sampling points per subject, and sampling points as defined by the authors (Table S2). Such diversity in research design has greatly increased the variance in observed effects in the studies. The differences in data presentation (i.e., relative abundance, absolute abundance, presence/absence, and specific units of measurement used) used in different studies also further narrowed the number of studies that passed the eligibility criteria, result-

Table 2b. Abundance of major antibiotic resistance genes (ARGs) across various surface waters detected through quantitative PCR (qPCR) (copies/16srRNA)

Antibiotic Class	Resistance Genes	Concentration (copies/16srRNA)	Reference
Beta-lactam	*blaTEM*	5.14×10^{-3} to 6.68×10^{-1}	Guan *et al.* 2018
Macrolide	*ermB*	1.00×10^{-2}	Niu *et al.* 2016
	ermF	1.26×10^{-4} to 9.38×10^{-3}	Guan *et al.* 2018
Sulfonamide	*sul1*	2.81×10^{-5} to 1.04×10^{6}	Jiang *et al.* 2021; Mao *et al.* 2014; Chen *et al*. 2015; Na *et al.* 2014; Yang *et al.* 2017; Zhou *et al*. 2014
	sul2	1.04×10^{-5} to 9.46×10^{6}	Mao *et al.* 2014; Chen *et al*. 2015; Na *et al.* 2014; Yang *et al.* 2017; Zhou *et al.* 2014; Zhang *et al*. 2013
Tetracycline	*tetA*	1.00×10^{-5} to 1.00×10^{-4}	Yang *et al.* 2017
	tetB	1.00×10^{-5} to 1.00×10^{-4}	Yang *et al.* 2017
	tetC	1.00×10^{-5} to 1.32×10^{1}	Guan *et al.* 2018; Ling *et al*. 2013; Yang *et al.* 2017
	tetM	1.00×10^{-6} to 2.33×10^{1}	Chen *et al.* 2015; Niu *et al.* 2016; Yang *et al.* 2017; Zhou *et al*. 2014
	tetQ	1.00×10^{-5} to 1.00×10^{-4}	Yang *et al.* 2017
	tetT	1.20×10^{5} to 6.76×10^{4}	Mao *et al.* 2014
	tetW	1.58×10^{-6} to 8.34×10^{4}	Mao *et al.* 2014; Zhou *et al.* 2014
Quinolone	*qnrD*	1.52×10^{-5} to 7.33×10^{-5}	Yang *et al.* 2017

ing in a small sample size for the meta-analysis. Finally, the small sample size commonly used in the studies significantly reduced the statistical power of data (Nobrega *et al.,* 2020). Nonetheless, carefully following the PRISMA protocol and arriving at such findings, this study still provided insights into the status of ARG occurrence across surface waters and its implications, which are further discussed.

ARGs detected using high throughput methods (next-generation sequencing, ddPCR, and HT-qPCR)

Although conventional and qPCR have been the traditional methods employed in detecting and quantifying ARGs in samples, these methods are very limited in the number of ARGs that can be detected in a single experiment. Therefore, high-throughput methods such as HT-qPCR and next-generation sequencing methods (metagenomic and whole genome sequencing) are increasingly being applied in ARG monitoring and surveillance. Both HT-qPCR and next-generation sequencing methods can detect high numbers of ARGs in a single experiment (Table S3). Still, the targeted ARG subtypes varied for HT-qPCR and metagenomic studies. For the HT-qPCR studies, the highest detection rate was observed in Wen Rui Tang River where all 285 targeted ARGs were detected (N=17) (Zhou *et al.,* 2017), while the lowest detection rate was in Ying Lake, where out of 284 targets, only 59 ARGs were detected (N=3) (Gu *et al.,* 2019) (Table 3). A possible explanation of the low detection rate here could be the sample number. All 9 HT-qPCR studies included beta-lactamase and aminoglycoside resistance genes as their target ARGs. Also, the most frequently targeted ARGs include sulfonamide (8/9), tetracycline (8/9), vancomycin (6/9), macrolide-lincosamide-streptogramin B (MLSB) (5/9), and multidrug resistance genes (6/9). On the other hand, ARGs conferring resistance to fluoroquinolone (3/9), macrolide (3/9), florfenicol/chloramphenicol/amphenicol (FCA) (2/9), phenicol (2), and diaminopyrimidine (1/9) were sporadically studied.

Of the 14 sequencing-based studies reviewed, 13 targeted tetracycline. Additionally, aminoglycoside (12/14), phenicol (12/14), beta-lactam (11/14), and sulfonamide (11/14) were among the most frequently targeted ARGs. On the other hand, the least studied ARGs are those for aminocoumarin, aminonucleoside, cephalosporin, elfamycin, lincosamides, nitroimidazole, acridine dye, aminocyclitol, glycopeptide, glycylcycline, lantibiotic, mupirocin (monocyclic carbolic acid), nitrofurans, oxazolidinones, penicillin, pyrazine, pyridopyrimidine, tetracenomycin, and triclosan. Up to 816 subtypes of ARGs were detected in watersheds in China, although it must be noted that these are from the total number of samples obtained in rural, periurban, and urban watersheds. Compared with this study, the other studies reviewed fewer ARG subtypes, ranging from 17 to 277.

For both HT-qPCR- and sequencing-based studies, the presentation of data varied. Of the 9 HT-qPCR studies, 4 presented their data as absolute ARG abundance (Gao *et al.,* 2022; Zhou *et al.,* 2017; Cheng *et al.,* 2020;

Ouyang *et al.,* 2015), while 3 studies used relative ARG abundance to present their data (Huang *et al.,* 2022; Gu *et al.,* 2019; Zhou *et al.,* 2023). Meanwhile, both absolute and relative abundance were used to present ARG levels in one study (Zheng *et al.,* 2017). Of the 14 metagenomic studies, 4 presented their data as absolute ARG abundance (Jiang *et al.,* 2018a; Liu *et al.,* 2021; Lu *et al.,* 2018) while 5 studies presented their data as relative abundance (Davis *et al.,* 2020; Hou *et al.,* 2020; Liu *et al.,* 2019; Jia *et al.,* 2017; Yang *et al.,* 2022). Meanwhile, 4 studies only used the metagenomic sequencing method to detect the presence/absence of certain ARGs (Ogura *et al.,* 2020; Fresia *et al.,* 2019; Hatosy *et al.,* 2015; Quillaguamán *et al.,* 2021). One study presented the ARG abundance regarding frequency log read numbers (Zhang *et al.,* 2022). This varied way of presenting the data on the abundance of ARGs brought about difficulty in summarizing or pooling the data, hence, a challenge in visualizing the overall status of ARG abundance in surface water.

Common pathogens associated with the ARGs

Studies investigating the association between the abundance of ARGs and microbial communities present in the surface water samples were reviewed to see which groups of microorganisms are commonly associated with the ARGs in surface waters (Table S4). In most of the studies, proteobacteria were the potential hosts of the ARGs detected in surface water samples (Yang *et al.,* 2022; Wang *et al.,* 2018; Gu *et al.,* 2019; Zheng *et al.,* 2017; Zhou *et al.,* 2023). It is quite alarming that proteobacteria were the main potential carriers of the ARGs in the surface waters studied as proteobacteria are known to harbour most gram-negative bacteria such as *Escherichia coli* (carbapenem-resistant and ESBL producing), *Salmonella* spp., *Vibrio cholerae*, *Yersinia pestis*, *Helicobacter pylori*, and *Legionella*, which are included in the priority pathogens according to the World Health Organization (WHO 2017). The studies reviewed also demonstrated the diversity of other potential hosts of ARGs in surface water. For instance, *Burkholderia*, *Zooglea*, *Bacteroides*, and *Prevotella* were found to be the main hosts harboring the ARGs found in Wen Rui Tang River, China (Zhou *et al.,* 2017). *Phycisphaeraceae*, *Actinobacteriota*, and *Sporichthyaceae* were also found to be the main hosts of ARGs in Minjiang River, China (Huang *et al.,* 2022). However, the overall picture of the diversity of potential hosts of ARGs must be considered with caution because in some studies, although diversity in the potential hosts may be demonstrated, it must be noted that in these studies, the water samples came from multiple surface waters. For example, very diverse groups of *Proteobacteria*, *Lactococcus*, *Bacillus*, *Cloacibacterium*, *Hydrogenophaga, Polynucleobacter, Acidovorax, Sulfurospirillum*, and *Tolumonus* were the microbial groups associated with the ARGs investigated in three different rivers - Weihe River, Fenhe River, and Yellow River (Wang *et al.,* 2018).

Meanwhile, *Comamonadaceae*, *Flectobacillus*, and *Flavobacterium* were the main hosts of various ARGs investigated in various urban rivers in Japan (Kasuga *et al.,* 2022). The presence or diversity of the microbial communities influencing the ARGs in surface waters may be explained by various factors such as geographical location, variations in physicochemical properties of water (Wang *et al.,* 2018), and the possible influence of water treatment processes (Lu *et al.,* 2018) and discharge of wastewater in the receiving surface water (Jia *et al.,* 2017). The microbial community and ARG co-occurrence analysis tools used in the reviewed studies are mostly network analysis based on correlation methods and multivariate statistical techniques such as redundancy analysis (RDA) and mantel test. It must be noted that there is still a plethora of other computational methods that can be used to analyze microbial community and ARG co-occurrence patterns, and that existing methods vary in sensitivity and specificity. It can bring about some compositionality bias that may affect the profiling results (Matchado *et al*., 2021). There are no strict rules regarding which methods must be applied to a specific data set. In this specific matter, a review by Paliy and Shankar (2016) as well as Matchado *et al.* (2021) gives a specific discussion and comparison of the various methods. It provides suggestions for the proper selection of methods to be used.

Impact of anthropogenic factors on antibiotic resistance gene (ARG) abundance

Numerous studies have incorporated human-related factors into their research to identify connections within their findings. The presence and influence of antimicrobial resistance genes (ARGs) in surface water are contingent on the degree of urbanization in a specific area. Importantly, ARGs are often shared between human pathogens and environmental bacteria, and this sharing is facilitated, particularly in proximity to urbanized regions. Current data suggests that urban surface waters have higher ARGs compared to pristine environments like untouched rivers and lakes (Table 4). For instance, a study conducted by Liu *et al.* (2021) along China's Tsangpo River discovered intrinsic ARGs even in pristine river sections. As one approaches urban areas, the diversity of ARGs in the river increases, resulting in a wider range of ARG subtypes, including sulfonamides and tetracycline. Similarly, a study by Jiang *et al.* (2018a), focusing on China's Pearl River, identified two dominant genera, *Francisella* and *Bacillus*, in both natural environments and drinking water sources. These findings collectively highlight the dynamic nature of

Table 3. Antibiotic resistance genes (ARGs) in various surface waters detected through digital droplet PCR (ddPCR) and high throughput qPCR (HT-qPCR)

Surface water	Method	Number of occurred ARG sub-types	Number of target-ed ARGs	Data type	ARG abundance	ARGs detected	Reference
Liaohe River Daliaohe River Hunhe River Taizi River	HT-qPCR	164	295	Absolute abundance	2.18×10^4 to 3.95×10^7 cop-ies/L	MDR, Beta-lactam, Tetracycline, Aminogly-coside	Gao *et al.* 2022
Weihe River	ddPCR	6 (6)	6/6	Absolute abundance	1.32×10^5 copies/ mL	Beta-lactam, Vancomy-cin, Aminoglycoside	Wang *et al.* 2018
Wen Rui Tang River	HT-qPCR	285	285	Absolute abundance	3.61×10^{10} to 3.02 $\times 10^{11}$ copies/L	Aminoglycoside, Beta-lactam, Fluoroquino-lone, Macrolide, Sulfon-amide, Tetracycline, Vancomycin	Zhou *et al.* 2017
Linshan River Wujia Fishpond	HT-qPCR	163	283	Absolute abundance	5.96×10^7 to 6.69 $\times 10^9$ copies L	Florfenicol/ chloramphenicol/ amphenicol (FCA), Aminoglycoside, Beta-lactam, MLSB, MDR, Sulfonamide, Tetracy-cline, Vancomycin	Cheng *et al.* 2020
Jiulongjiang River	HT-qPCR	212	285	Absolute abundance	7.18×10^8 to 1.03 $\times 10^{11}$ copies L^{-1}	Vancomycin, Tetracy-cline, Sulfonamide, MLSB, Florfenicol/ Chloramphenicol/ Amphenicol (FCA), Beta-lactam, Aminogly-coside	Ouyang *et al.* 2015
Minjiang River	HT-qPCR	252	327	Relative abundance	83 ± 5 to $6.82 \times 10^6 \pm 2.63 \times 10^6$ copies/uL	Aminoglycoside, Mac-rolide-Lacosamide-Streptogramin B (MLSB), beta-lactam, fluoroquinolones, MDR, Phenicol, Sulfonamide, Tetracycline, Diamino-pyrimidine, Vancomycin	Huang *et al.* 2022
Ying Lake	HT-qPCR	59	284	Relative abundance	5.59×10^{-6} to 2.30 $\times 10^{-2}$ cop-ies/16SrRNA copy	Sulfonamide, Tetracy-cline, Vancomycin, MLSB, Aminoglycoside, Beta-lactam, MDR	Gu *et al.* 2019
Rivers near Kathmandu Valley	ddPCR	12	13	Relative abundance	4.2 log10 - 9.3 log10 copies/100 ml	Sulfonamide, Tetracy-cline, Aminoglycoside, Beta-lactam, Quinine Tetracycline, Diamino-pyrimidine, Quinolone, Vancomycin, Methicillin	Amarasiri *et al.* 2022
Unnamed aqua-culture ponds in Baiyun, Guang-dong China	HT-qPCR	89	133	Relative abundance	-	Aminoglycoside, Beta-lactam, Phenicol, Mac-rolide, Sulfonamide, Tetracycline, MDR	Zhou *et al.* 2023
East Tiaoxi River	HT-qPCR	236	285	Absolute abundance (copies/L) and rela-tive abun-dance (copies/ cell)	absolute abun-dance: 6.1 x 10^8 to 2.1 x 10^{10} copies/L relative abun-dance: 0.033 to 0.158 copies/cell	Aminoglycoside, Beta-lactam, Fluoroquino-lone, Macrolide, Sulfon-amide, Tetracycline, Vancomycin	Zheng *et al.* 2017
Urban Rivers in Japan	HT-qPCR	9-53	67	Pesence/ absence	N/A	Aminoglycoside, Sul-fonamide, Beta-lactam, Macrolide-Lincosamide - StreptograminB (MLSB), MDR	Kasuga *et al.* 2022

Table 4. Summary of findings regarding antibiotic resistance genes (ARG) abundance of pristine vs. anthropogenically impacted areas

Surface Water	Quantification Method	Highest Mean ARG Abundance	Impacting Factors	Overall Findings	Reference
Yarlung Tsangpo River	metagenomic sequencing	0.55 to 1.34 copies/cell	urbanized areas; dam-regulated activities	Constant relative abundance along pristine, urbanization, and dam-regulated areas but ARG diversity was impacted by anthropogenic activities	Liu *et al*. 2021
Pearl River Delta Region	metagenomic sequencing	0.55 to 1.34 copies/cell	livestock farms; aquaculture farms; wastewater treatment plants; pharmaceutical industries	Higher ARG abundance in downstream sites.	Jiang *et al.* 2018a
Weihe River	ddPCR	1.32×10^5 copies/mL	urbanized areas	ARGs were relatively high in sampling sites located in urban areas compared to rural areas	Wang *et al.* 2018
Minjiang River	HTPCR	6.82×10^6 copies/uL	aquaculture; dam construction; urban activities	More diverse ARGs in urban river waters compared to reservoir water.	Huang *et al.* 2022
Ying Lake	HTPCR	2.30×10^{-2} copies/16SrRNA	aquaculture	ARGs were generally higher in the fish farming area than in the upstream region.	Gu *et al.* 2019
Jiulongjiang River	HTPCR	1.03×10^{11} copies/L	urbanized area; highly populated areas	ARGs in urban samples were over two orders of magnitude higher than the pristine samples	Ouyang *et al.* 2015
Various Surface Waters in the Kathmandu Valley	ddPCR	9.3 log10 copies/100 ml	agriculture farms	Exposed water samples have higher ARGs.	Amarasiri *et al.* 2022
Liaohe River Daliaohe River Hunhe River Taizi River	HTPCR	3.95×10^7 copies/L	animal husbandry; aquaculture; urbanized areas	The ARGs in urban sites have almost the same abundance as in rural sites	Gao *et al.* 2022
Wen Rui Tang River	HTPCR	3.02×10^{11} copies/L	wastewater; urbanized areas	Urban sites have higher ARGs compared with upstream waters	Zhou *et al.* 2017

bacteria hosting various antimicrobial resistance genes (ARGs), with their abundance either increasing or decreasing depending on specific environmental conditions. Consequently, this variability can potentially affect the prevalence and distribution of ARGs, as demonstrated in the study of Yang *et al.* (2014).

Mobile genetic elements (MGEs) are also being studied to indicate human-related pollution. A key gene used for this purpose is *IntI1*, also known as the class 1 integron-integrase gene. It was selected as a marker due to its association with genes that confer resistance to antibiotics, disinfectants, and heavy metals (Liebert *et al.,* 1999; Partridge *et al.,* 2001). The abundance of this gene can rapidly change in response to environmental pressures, including urbanization, and it is found in various pathogenic and non-pathogenic bacteria in both humans and animals (Gillings *et al.,* 2014). The research demonstrated that *IntI1*, owing to its broad detection capabilities, facilitates the horizontal transfer of other clinically significant ARGs, such as *blaTEM* and *aadA* genes, which confer resistance to specific antibiotics (Gillings *et al.,* 2014; Zhang *et al.,* 2011). This finding is supported by the data from the study of Wang *et al.* (2018), which showed high levels of antibiotic resistance for beta-lactams and streptomycin in all the water samples obtained from Weihe River in 2015. Moreover, *IntI1* exhibited an absolute abundance ranging from 2.68×10^3 to 1.20×10^5 copies/mL. The study's use of *IntI1* as a gauge for anthropogenic activities found that the gene promotes the transfer of other important ARGs, thereby contributing to antibiotic resistance in the environment (Wang *et al.,* 2018).

Another human-induced factor affecting the fluctuations of antimicrobial resistance genes (ARGs) in surface water is the construction of dams. The impact of dam construction on ARGs is distinct from the consequences of urbanization, as evident from the clear categorization of their collected samples. In areas regulated by dams, the prevalence of ARGs remains notably high, accompanied by a significant increase in ARGs associ-

ated with chloramphenicol and aminoglycoside resistance. This contrast is particularly noticeable when compared to the composition of ARGs in urbanized regions (Liu *et al.,* 2021). This suggests that dam construction can amplify or diminish the exposure of ARGs associated with human pathogens, depending on specific regulations and activities in the area. Additionally, aquaculture represents another anthropogenic factor influencing the abundance of ARGs in surface waters. Gu and colleagues explored the impact of various fish farming methods on ARG abundance in Ying Lake, China. The results of Gu *et al*., (2019), derived through qPCR, revealed that specific fish farming techniques, such as box-type fish farming, can intensify the dissemination of more ARGs. This effect is particularly pronounced when aquacultural treatments, like benzyldimethyldodecylammonium chloride, enrich specific ARGs, such as *blaCTX-M* and *tetA*. In summary, this research concludes that certain aquacultural practices tend to escalate the presence of ARGs in surface water (Gu *et al.,* 2019).

ARGs in surface water and its indirect impact to human health

ARGs present in surface waters, either suspended as extracellular DNA, or carried within viable cells, can be acquired by susceptible microorganisms in that environment, thereby demonstrating the critical role of surface waters in disseminating ARGs. The presence of ARGs in microorganisms may be intrinsic or acquired via spontaneous mutations that occur during bacterial replication or via horizontal gene transfer (HGT) through transformation, transduction, or conjugation mechanisms (Amarasiri et al., 2020). Some water-dwelling bacteria intrinsically carry antibiotic resistance genes, such as several species in the genus *Aeromonas* and *Pseudomonas* (Santajit et al. 2016). A study also demonstrated the presence of *blaTEM* in the non-pathogenic strains of the genus *Serratia* isolated from freshwater environments (Navarro et al., 2023). Genomic analysis also showed that ARGs in some medically important pathogens originated from pathogenic bacteria in the aquatic environment (Suzuki et al. 2017). Surface waters, unlike groundwater, are exposed to the atmosphere and are affected by water draining from surrounding lands (Katsanou and Karapanagioti 2019). They are also highly impacted by wastewater from both domestic and industrial systems, such as pharmaceutical plants, hospital waste, and food production systems, which are environmental hotspots for antibiotic resistance (Gwenzi et al. 2020; Obayiuwana et al. 2021; Rozman et al. 2020; Tiedje et al. 2023; Cho et al. 2020). These provide ideal conditions for disseminating ARGs through the various mechanisms mentioned. This can lead to increased antibiotic-resistant pathogenic bacteria in surface waters, which can ultimately cause food and waterborne diseases. Infections caused by antibiotic-resistant pathogens may cause disease outbreaks, as antibiotic resistance confers enhanced virulence and transmission. The increased pathogenicity also leads to poorer disease outcomes (i.e., increased case fatality and longer disease duration) (Rödenbeck et al., 2023).

Furthermore, due to the disparities in the healthcare system among countries, AR impacts can be more apparent in developing nations. Such inadequacies can be seen in healthcare facilities, in the availability of medications, and in elevated disease morbidity, which further demands more antibiotic use (Gwenzi et al., 2020). However, further risk assessment must be done to investigate the true impact of ARG in surface waters on human health.

Conclusion

Most studies on ARG detection and quantification in surface waters obtained globally were from China and focused on rivers. PCR-based and sequencing-based methods were the main methods employed in the studies. The highest abundance of ARG in this review was 1.00×10^{11} copies/mL. Sulfonamide and tetracycline resistance genes were the most frequently targeted genes in the qPCR studies. Meanwhile, vancomycin resistance genes had a high detection rate, raising concern. Factors that mainly affect ARG abundance in surface water include urbanization and domestic wastes, aquaculture farming, construction of dams, and wastewater, among others. Ultimately, ARGs in surface waters may contribute to increased AR pathogens causing foodborne and waterborne diseases. High-throughput sequencing methods such as HT-qPCR and metagenomic sequencing effectively identified and quantified many ARG subtypes in surface water samples. The ARGs in the reviewed studies were mostly associated with Proteobacteria.

Recommendations

China is rich in major rivers flowing throughout the country and to 18 neighbouring countries. Water resources with such wide geographical and economic coverage must be monitored for relevant matters such as AMR dissemination. Being an upper middle-income country, as defined by the World Bank as of 2023, China can indeed support various research and development projects. Hence, most of the studies included in this review were from China. This serves as a wake-up call for other nations, particularly for countries like the Philippines, which are also highly dependent on water resources economically, to partake in AMR surveillance studies and for the government to provide more funding and support towards such research endeavours.

This study attempted to conduct a meta-analysis on the

abundance of ARGs in surface waters detected through qPCR; however, factors identified, which include the differences in research design, differences in data presentation, and small sample size, lead to a high heterogeneity between the studies. Therefore, the pooled ARG abundance was not obtained. This indicates a need for a conventional manner of presentation of the data for ARG abundance.

Computational methods that can rapidly analyse the large set of data produced through high throughput methods aid in the correlation of ARGs with the microbial communities present, identify their potential hosts, and correlate which specific anthropogenic factors greatly impact ARG abundance. However, considering the costs of each analytic method and the variable research capabilities of different countries, the applicability of PCR-based and such high throughput methods in AMR surveillance must still be further developed and systemized to optimize their utility and applicability depending on the needs and economic setting of the country.

Supplementary Information

The authors are responsible for the content or functionality of any supplementary information. Any queries regarding this should be directed to the corresponding author. The supplementary information is downloadable from the article's webpage and will not be printed in the print copy.

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Conflict of interest

The authors declare that they have no conflict of interest.

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