

## **EMERGING ANTIBIOTIC RESISTANCE GENES IN THE AQUATIC ECOSYSTEMS: A REVIEW**

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### **ABSTRACT**

Antibiotic use may hasten the emergence of antibiotic resistance genes (ARGs) in bacteria, posing health concerns to humans and animals alike. The emergence of ARGs in aquatic ecosystems is becoming a growing problem around the world. Hundreds of different ARGs encoding antibiotic resistance have been discovered in bacteria found in sewage, effluent treatment facilities, surface water, groundwater, and even drinking water. Antibiotic resistance genes can be transmitted from one cell to another by conjugation, transformation, or transduction. This gene exchange allows resistance to spread quickly within a bacterial community and between various species of bacteria. This is more so in an ideal environment such as aquatic ecosystems. The purpose of this article is to review the presence of ARGs in various aquatic ecosystems and how this will affect humans. Based on 30 selected studies in the last five years (2017-2022), we notice that ARGs can be found in various aquatic ecosystems, including surface water, wastewater, and plastic and microplastic debris found in these ecosystems. There are some studies showing ARGs are directly affecting humans, but there are also many ongoing efforts and technologies to reduce ARGs in aquatic ecosystems. From the discussion, we conclude that antibiotic resistance genes can thrive in various aquatic ecosystems, which offer an ideal environment for their replication. Although aimed at reducing pollutants in the environment, wastewater treatment plants (WWTPs) have been identified as potential hubs for the spread of antibiotic resistance determinants into aquatic ecosystems, showing the importance of continuous monitoring and finding solutions.

**Keywords:** Antibiotic resistance genes (ARG); antibiotic resistance bacteria (ARB); microplastics; aquatic ecosystems; antibiotics

## Introduction

Antibiotics in modern medicine date back to the discovery of penicillin as an antibacterial agent by Alexander Fleming in the late 1920s. Since then, antibiotics with various structures have been discovered and currently are commonly divided into 16 families with different chemical structures, including  $\beta$ -lactams, tetracyclines, sulfonamides, aminoglycosides, fluoroquinolones, macrolides, trimethoprim, and glycopeptides (Zhuang et al., 2021). One of medicine's biggest successes was the discovery and synthesis of antibiotics in the previous century. Human morbidity and mortality have decreased as a result of the usage of antimicrobial drugs, which has contributed significantly to an increase in human life duration. However, although antibiotics have been regarded as one of the most important discoveries in modern medicine, the time has proven that the continuous and excessive use of antibiotics has caused the spread of antibiotic resistance bacteria (ARB) and become a serious concern to the medical communities. The exposure of patients to the antibiotic resistance genes (ARGs) carried by these pathogenic ARBs caused antibiotics to be ineffective for bacterial treatment.

ARB and ARGs occur naturally, but pressures imposed by certain human activities have increased their dissemination in the environment. The intensive use of antibiotics in agriculture, farming, aquaculture, and medical treatments has caused some ARBs to evolve and become superbugs or emerging pathogens (Zhuang et al., 2021). The ARGs can be transferred traditionally from parent ARB to their offspring. However, more commonly, the ARGs are transferred through horizontal gene transfers where the genetic factors can be transferred from an individual bacterium to another individual bacteria from the same genus and even individual bacteria from different genera via conjugation, transduction, transformation, and other methods (Dong et al., 2021).

In addition to the concern in clinical settings, the emergence of ARB and ARGs from various sources has also become an environmental concern because they accumulate in water, soil, and air (Zhuang et al., 2021). The occurrence of antibiotics and antibiotic resistance genes in various aquatic ecosystems has been widely studied including in rivers (Arsand et al., 2020; Ding et al., 2021; Reichert et al., 2021), estuaries (Guo et al., 2020; Zhou et al., 2022), coastal areas (Di Cesare et al., 2021; Su et al., 2020) and freshwater lakes (Yang et al., 2017) showing the great importance of the subject. The presence of ARGs in these aquatic ecosystems will later infiltrate the food chain and also gain access to the human body (Li et al., 2021).

This review looks at selected current research articles that focus on the presence of antibiotic resistance genes in various aquatic ecosystems. The Scopus database was used to gather these articles. We selected 30 latest articles that are related to the objective of our review paper. We are interested to see the types of ARGs commonly studied or found within aquatic ecosystems, their sources, impacts, and current technology to eliminate these ARGs in the environment. Information from this review is hoped to develop more knowledge about how ARGs spread or disseminate in our ecosystems. Other than that, this review is also hoped to raise awareness of the available technologies to be supported in reducing the abundance or concentration of antibiotics as well as ARGs in aquatic ecosystems.

## The Presence and Sources of Antibiotic Resistance Genes (ARGs) in the Aquatic Ecosystems

Antibiotic resistance bacteria (ARBs) found in various aquatic ecosystems have become an emerging issue as they are highly transmissible and easily affect the living organisms and those interacting with them, including humans. Previous studies have focused on several types of aquatic ecosystems to see the presence of ARGs. These include natural water bodies (Arsand et al., 2020; Guo et al., 2020; Yang et al., 2017) and wastewater treatment plants (WWTPs) (Bengtsson-Palme et al., 2019; González-Plaza et al., 2019; Lv et al., 2020; Reichert

et al., 2021; Su et al., 2020). There are also many studies that look at the bacterial assemblages, including ARBs on plastic debris from these aquatic ecosystems, as they are expected to increase the risks of ARGs dissemination (Di-Cesare et al., 2021; Guo et al., 2020; Yang et al. 2019).

### ***ARGs in natural water bodies***

Studies on ARGs in various aquatic ecosystems usually focus on quantifying the presence of antibiotics before further analysis is done to determine ARGs conferring resistance to the antibiotics. Different studies focus on different types of antibiotics and ARGs based on their high detection frequency and abundance or based on the background information of the ecosystem in their study. For instance, Arsand et al. (2020) quantified 40 antibiotics of 5 different classes from the water samples of Dilúvio River, southern Brazil. In the study,  $\beta$ -lactam (cephalexin), quinolones (ciprofloxacin and norfloxacin), macrolides (azithromycin and clindamycin), sulfonamides (sulfadiazine and sulfamethoxazole), and trimethoprim were the compounds present in all samples. Four ARGs conferring resistance to sulfonamides (sulI),  $\beta$ -lactams (blaTEM), erythromycin (ermB), and quinolones (qnrS) were also analysed and these ARGs are found in all samples with the highest concentration found for blaTEM. Antibiotics and ARGs considered in the study were all related to the pharmaceutical products commonly used in Brazil. Another study examined tetracyclines, beta-lactams, macrolide, lincosamide and streptogramin (MLS) antibiotics, sulfonamides and fluoroquinolones. The researchers found selected genes encoding resistance to them in the Pilica River, Poland (Koniuszewska et al., 2020). The study reported that selected genes (bla(TEM), tet(A), tet(E), ermF, linA) were present in all or nearly all water samples regardless of sampling date or sampling site, suggesting important genes to be studied in similar studies. Meanwhile, Komijani et al. (2021) decided on the antibiotics and ARGs to be considered in their studied lakes in Iran by first conducting a meta-analysis of 150 previous studies. Among the antibiotics and ARGs commonly studied, it was found that tetC was the most prevalent in 5 of the 6 lakes sampled. The frequency of quinolone resistance, b-lactam resistance, and tetracycline resistance overall was 38%, 34% and 28% in all lakes, respectively.

From the studies, we can see that different ARGs are prevalent in different areas. There are many factors contributing to this. The study by Arsand et al. (2020) suggested that the quantity of each antibiotic class agrees with the pharmaceuticals often used by the Brazilian population. The study by Komijani et al. (2021) relates the higher abundance of b-lactams, quinolones and tetracyclines to the intense industries and other human activities in some of the lakes. Meanwhile, Koniuszewska et al. (2020) suggested that the different abundances are correlated to seasons. From these examples, it can be concluded that the abundance of ARGs can be affected by spatial or temporal variables. A study on coastal waters along the coastline of China found a higher absolute abundance of ARGs during winter, which is  $1.39 \times 10^5$  copies/mL compared to  $8.79 \times 10^4$  copies/mL during summer (Lu, Zhang & Wu, 2020). The study by Koniuszewska et al. (2020) in Pilica River, Poland, also reported that ARGs were most prevalent and diverse in their winter samples. It was suggested that the temporal variations may be due to infections and high drug consumption during the season. Meanwhile, in a more recent study by Zheng et al. (2022), it was reported that the abundance, richness, and diversity of ARGs in the dry season exceeds those in the wet season for China's estuaries. The study by Zheng et al. (2022) also suggested that temperature is a major contributing factor to the persistence of antibiotic resistance based on many previous studies. The temporal distribution and abundance of ARGs perhaps not only depends on the temperature but also other natural factors. However, studies have shown that climate and human activities are the major drivers of ARGs abundance on a large scale. Considering spatial and temporal variations, a long-scale study in Yangtze Estuary was done by Guo et al. (2020) to see the different abundance of ARGs between periods and found that the abundance of ARGs, including sul1, sul2, tetM,

tetW, aac(6)-Ib and qnrS, was higher in recent years (from 2015 to 2019) than that in earlier years (from 2007 to 2011) (Guo et al., 2020). This can be contributed by both changing climate and changes in land used.

Beside spatial and temporal factors, the distribution of ARGs can also be influenced by other surrounding components. Unexpectedly, the distribution of ARGs is not necessarily correlated to their respective antibiotics. A study investigated the pollution and ecological risks of 39 antibiotics in Xiong'an New Area (XANA), China, and found that only some of the ARGs are significantly positively correlated to the abundance of related antibiotics. The study suggested that antibiotics do not always increase the expression of ARGs, but sometimes might inhibit the expression of corresponding ARGs (Fu et al., 2022). Li et al. (2015) look at sulfonamide resistance genes in surface water samples from Daliaohe and Liaohe river estuaries to understand its mechanism and suggest that the abundance of sul-ARGs is not correlated to the concentration of sulfonamides. However, the propagation of sul-ARGs was actually facilitated by another ARG which is class 1 integrons (int1) in estuaries. The abundance of antibiotics and ARGs are not always correlated because the interactions in the aquatic ecosystems can be complex. Instead of antibiotics, the quantity of ARGs is also commonly correlated with other factors, including metals and metals resistance genes (Guo et al., 2020; Komijani et al., 2021; Li et al., 2021; Zhao et al., 2021).

Most of the studies on the ARGs distribution usually focus on water samples (Arsand et al., 2020; Koniuszewska et al., 2020; Yang et al., 2017). However, some other studies look at sediment samples as it is argued that antibiotics and associated ARGs will mostly sink on the bottom of the water bodies and the quantification can be more accurate based on sediment samples. For instance, a study by Fu et al. (2022) reported the total antibiotic concentrations in the circulation system of water, such as surface water, groundwater, and sediment were 12.71–260.56 ng/L, ND–196.12 ng/L, and 38.03–406.31 ng/g, respectively. While the study found the highest concentration of ARGs in sediments, another study that looks at sediment samples of 15 lakes from the middle and lower reaches of the Yangtze River, China found that sediment characteristics (moisture, density, total nitrogen, total carbon, ammonium, and nitrate) have no significant effect on ARGs distribution. Continuous studies on factors driving the abundance of ARGs are needed for further understanding.

In addition to understanding how ARGs are accumulated locally, it is also important to consider external factors. For instance, a study suggested that ballast water was an important moving carrier for the global transfer of ARGs. The study found that ballast water from 28 vessels sailing to Shanghai and Jiangyin (China) had considerable levels of ARGs, and integrase of the class-I integrons (int1) were detected in all ballast water samples. sul1 and tetQ were the most and least abundant ARGs in ballast water samples, respectively. Moreover, the bacterial hosts of ARGs were generally different in the unexchanged ballast water (UEBW) and exchanged ballast water (EBW) (Lv et al., 2020). This study shows the importance of considering the outside sources of ARGs in finding solutions to the issue.

### ***ARGs in wastewater treatment plants (WWTP)***

While the presence of ARGs in natural water bodies can be worrying, there is a more urgent need to find a solution to remove ARGs from wastewater treatment plants. More studies began to focus on the risks of ARGs in WWTPs as they have been proposed as hot spots for the dissemination of antibiotics and antibiotic resistance determinants into the aquatic environment (Bengtsson-Palme et al., 2019; González-Plaza, et al., 2019; Reichert et al., 2021; Su et al., 2020). Biological treatments that aim to reduce pollutants can result in massive enrichment of antibiotic resistant bacteria and resistance genes and, consequently, alteration of the sludge microbial community due to selection by the antibiotic residues (Bengtsson-Palme et al., 2019).

There are a few studies that compare wastewater samples from different points of a treatment plant to show how WWTP can increase the presence of ARGs. For instance, a study in Southern Germany used qPCR to detect the existence of 13 ARGs and seven gene indicators for facultative pathogenic bacteria (FBP) in sampling sites downstream and upstream of a small WWTP. The results show that the abundance of ARGs was higher in water samples collected downstream of the WWTP than in those collected upstream of the WWTP. Some ARGs can only be detected downstream, including a very important ARG, which is *mcr-1* (Reichert et al., 2021). Another study shows that the relative abundance of resistance genes [*sul1*, *sul2*, *qacE/qacE1*, *tet(A)*], class 1 integrons (*intl1*), and *IncP-1* plasmids increased dramatically in receiving sediments compared to the upstream sediments of two antibiotic production facilities in Croatia (González-Plaza, et al., 2019).

The relative abundance of ARGs in WWTP is highly influenced by the industries feeding the influent. A study by Bengtsson-Palme et al. (2019) compares sludge samples from a WWTP receiving wastewater from a Croatian azithromycin manufacturing facility to sludge from a WWTP located in Zagreb, which receives mainly municipal wastewater. They found that the abundance of antibiotic resistance genes was three times higher in sludge from the treatment plant receiving wastewater from pharmaceutical production than in municipal sludge from a sewage treatment plant in Zagreb. There is also another study that compares the abundance of ARGs between WWTPs, two effluent receiving locations, near Hangzhou Bay, China. The study found that two integrated wastewater treatment plants received more ARGs than pharmaceutical wastewater treatment plants but managed to eliminate ARGs more efficiently than the other two pharmaceutical wastewater treatment plants (Su et al., 2020). Besides showing the impact of industry on the relative abundance of ARGs, the study by Su et al. (2020) also shows that different treatment activities can contribute to the reduction of ARGs, showing the importance of technology in controlling the issue.

### ***ARGs on plastic debris***

The presence of plastic debris in various aquatic ecosystems is suggested as a reservoir for antibiotic resistance genes and a potential vector for antibiotic-resistant bacteria (Di-Cesare et al., 2021). For instance, Reichert et al. (2021) studied the different amounts of ARGs in water, sediment and biofilm. The study found that some ARGs were higher in biofilm than in water and sediment samples. It is proven that some substrates found in aquatic environments can become a carrier for antibiotic resistance bacteria and associated ARGs. There are many other examples, including the study by Guo et al. (2020) that reported the average absolute abundances of most tested ARGs on the different types of plastic were generally higher than those in the sediment and water of Yangtze Estuary (Guo et al., 2020). In another example, both the Shannon-Wiener indices and richness of ARGs and multi drugs resistance genes (MRGs) in plastic microbiota were significantly greater than those of ARGs and MRGs in seawater microbiota in the North Pacific Gyre (Yang et al., 2019). Even a study of bioplastic found that bioplastic, that is often regarded as a more environmentally friendly product can also become the carrier for the ARGs in the aquatic ecosystems. A total of 182 ARGs from 19 different types were found in the samples, with an average of 74, 71, 70, and 75 distinct ARGs for seawater, ceramic, PET, and PHA samples, respectively (Di-Cesare et al., 2021).

On a more worrying note, ARBs and ARGs can be transferred very far from their sources when attached to plastic debris. A study looks at the biofilm communities of plastic debris that managed to reach King George Island (South Shetlands, Antarctica). Their tests on the biofilm showed multiple antibiotic resistances against the molecules cefuroxime and cefazolin (belonging to cephalosporins), cinoxacin (belonging to quinolones), and ampicillin, amoxicillin + clavulanic acid, carbenicillin and mezlocillin (belonging to beta-lactams) (Laganà et al., 2019).

Besides comparing the abundance of ARGs on plastic debris and their surrounding water, there is also a study that showed that ARGs on microplastics can be 103 times higher than those in water in the recirculating aquaculture system where ARGs are expected to be high. The study suggested that microorganism in biofilms favours the flow between surface-attached bacteria more compared to pathogens in water, thus facilitating the specific pathogens to acquire ARGs (Sun et al., 2021). Also, organic matter adsorption to microplastic particles boosted plasmid transfer frequencies, mirroring expected natural activities under conditions of high dissolved organic carbon, as demonstrated for natural organic matter aggregates (Arias-Andres et al., 2018). The study demonstrated that plasmid transfer was uniformly more favourable in natural lake communities established on microplastics compared to free-living bacteria or bacteria on natural aggregates. Microplastics can selectively enrich pathogens and specific ARG subtypes (Zhao et al., 2021). Zhao et al. (2021) demonstrated that heavy metals such as copper (Cu) and Zinc (Zn) can be absorbed by microplastics from the surroundings. The co-contamination of heavy metals and antibiotics will drive the co-selection of ARGs, MRGs, and the emergence of multidrug-resistant bacteria.

More currently, during the transmissible pandemic, a new concern arises with the high usage of disposable masks. A study investigated the abandoned mask to find out whether it has a high possibility of increasing the environmental threat from plastic contamination as the masks were made from non-biodegradable polymers such as polypropylene, polyethylene, and polyacrylonitrile. At Xinglin Bay, an estuary on the Taiwan Strait, two types of masks, activated carbon (C) and surgical (S) masks, were exposed to brackish water for 30 days, and samples were taken every seven days (Zhou et al., 2022). After 30 days, a total of 216 ARGs and 11 MGEs were found in the water and mask sample. Those ARGs came from nine different classes, which were Aminoglycoside, Beta-Lactam, Chloramphenicol, Macrolide lincosamide streptogramin B resistance (MLSB), Multidrug, Sulfonamide, Tetracycline, Vancomycin, and others. Using a Scanning Electron Microscope (SEM), it was shown that the index of Shannon bacteria was significantly higher for both mask types than in the water sample. This implies that discarded masks in marine systems are likely microbial shelters that provide a stable environment for microbial survival and propagation (Zhou et al., 2022). However, it was also recorded that the relative abundance of ARGs and MGEs in water samples was substantially higher than in either mask type at each sampling ( $P < 0.05$ ).

### **The Dissemination of ARGs and Potential Exposure to Human**

Studies discussing the presence and sources of ARGs in various aquatic ecosystems are very important as they may affect other living organisms through direct or indirect interactions. One study analyses the distribution of ARGs, including the tetA gene, sul2 gene, and kan gene, in Chironomidae larvae. The study demonstrated that Chironomidae larvae enriched ARGs from water and passed them on to downstream predators in the food chain mediated by conjugative transfer. Gram-negative bacteria such as Escherichia in Proteobacteria and Flavobacterium in Bacteroidetes are the primary host bacteria of ARGs. It has been confirmed by feeding experiments that ARGs from Chironomidae larvae can be enriched in the fish gut (Ding et al., 2021). This is an example of how ARGs can be transferred indirectly through trophic relationships up to the top predators, including humans.

Besides that, there are at least two other studies that look at direct exposure of ARGs to humans. For instance, a study investigating the distribution of antibiotic resistance genes (ARGs) and bacterial communities in swimming pools proved that swimming pools contained a higher relative abundance of 0.62 gene copies/16S rRNA and the absolute abundance is  $6.57 \times 10^8$  gene copies/L for ARGs on total average. The predominant types of genes that confer resistance to multidrug, aminoglycoside, and beta-lactam accounted for more than 85.10% of the total relative abundance on average. Some ARGs that were clinically related

were prevalent across different samples. Genes encoded with aminoglycoside (*aadA*, *aac*), beta-lactam (*blaOXY-1*, *blaGES*), and sulfonamide (*sul1*) were the most common in all swimming pools, which accounted for 23% of the total average. Besides that, the main exposure route of ARGs was estimated as dermal contact. After further analyses on humans were done, 18 ARGs that were not detected before swimming were found on human skin and remained after showering (Shuai et al., 2021).

Another study has investigated the presence of several ARGs, namely, *ermB*, *sul1*, *tetA*, *tetW*, *tetX*, and *mecA*, for resistance to sulfonamides, erythromycin, tetracycline, and methicillin antibiotics, respectively in the portable water catchment. In the raw source water before treatment and distribution, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* were found. Moreover, antibiotic resistance genes, like *tetA* and *sul1* were also observed in the raw source water, suggesting the presence of ARGs and ARB. *TetA* was more commonly found compared to *sul1*. *TetA* is one of the more common tetracycline resistance genes; thus the greater abundance of *tetA* compared to the other ARGs investigated is not surprising. The widespread use of tetracycline as a growth promoter in animal farms and the wide variety of bacterial species that have been shown to possess this gene also explain the abundance of *tetA*. However, there was no presence of ARGs or ARB in the treated and distributed water (Bergeron et al., 2017). It shows the importance of treatment technologies in reducing the occurrence of ARGs.

### **Current Efforts and Technologies to Reduce ARGs in The Environment**

As the presence of ARGs and ARBs has become more prevalent in the aquatic ecosystems, so have the efforts and technologies to remove them. Although various treatments have been studied and adapted, the efficiency of each treatment is still under study. Riaz et al. (2019) and Sabri et al. (2021) elaborated on the various technologies that include physical approaches (adsorption, membrane filtration, photolysis, and disinfection of AMR/ARGs by UV radiation), chemical treatments (oxidation: ozonation, chlorination), and biological treatments (aerobic digestion, anaerobic treatments, constructed wetlands).

While the studies by Riaz et al. (2019) and Sabri et al. (2021) focus on the treatment of ARGs and ARBs in the WWTP, many more studies focused on the biological treatment options although mostly are still studied under clinical laboratory setting. For instance, a study investigated the performance efficacy of vermifilter (VF) and found that earthworms and the VF-related microbial community reduced biochemical oxygen demand (BOD) and chemical oxygen demand (COD) by 78–85%, removed coliforms and pathogens by >99.9%, and induced a significant shift in the prevalence pattern of ARB. Molecular profiling of resistance-causing genes including ESBL (*blaSHV*, *blaTEM*, and *blaCTX-M*), MRSA (*mec-A*), and Colistin (*mcr-1*) validated the resistance pattern's likely causes (Arora et al., 2021).

In another study, easy-to-biodegrade food has been reported to improve the removal of toxic pollutants (Zhang et al., 2021). The study investigated whether such co-substrates may also decrease the abundance of ARGs and their transferal. Using a sequencing biological reactor, amoxicillin (AMO) degradation was examined utilising a co-substrate of 0–100 mg/L acetate sodium. Proteobacteria, Bacteroidetes, and Actinobacteria were identified as dominant phyla for AMO removal and mineralisation. The study reported that the total abundance of ARGs decreased by approximately ~30%, and the proportion of the most dominant antibiotic resistance bacteria, Proteobacteria, decreased by ~9%. The total abundance of plasmids that encode ARGs decreased by up to 30%, meaning that the risk of ARG dissemination was reduced. The study summarised that in an activated sludge process, easy-to-biodegrade food led to the simultaneous removal of antibiotics and ARGs (Zhang et al., 2021). It was suggested that easy-to-biodegrade food that acts as a co-substrate provides energy and becomes an

electron donor to improve the mineralisation of antibiotics while optimising bacterial community structures and altering the abundance of functional genes (Zhang et al., 2021).

Another study by Liu et al. (2021) investigated the effects of co-composting swine manure with different inoculants dominated by *Phanerochaete chrysosporium* (p), *Aspergillus niger* (a), and *Bacillus licheniformis* (b) on the simultaneous removal of multiple antibiotics and resistance genes as well as the evolution of the bacterial community. The highest removal extents of tetracycline and oxytetracycline were found in pile D (p:b:a=1:5:5, biomass), reaching 89.2% and 87.8%, respectively, while the highest removal extents of doxycycline and enrofloxacin were found in pile A (p:b:a=1:0:0, biomass), reaching 98.6% and 89%, respectively. Compared with the levels in pile B (control check), in pile D, ARGs, except those for sulfonamides, decreased by  $1.059 \times 10^{-3}$ – $6.68 \times 10^{-2}$  gene copies/16S rRNA copies (Liu et al., 2021). Meanwhile, Pruden et al. (2013) suggested a less technology-reliance technique, that is the containment of animal wastes for containing ARGs in manure, with additional benefits like nutrient management and soil and water quality protection. Improved manure collection and storage capacity can help to reduce surface run-off by allowing manure to be applied to land only when crop demands for water and nutrients are high (Pruden et al., 2013).

From various studies discussed, we suggest that physical and chemical treatments are commonly applied in WWTPs. However, they may not necessarily be targeted to reduce the amount of ARGs and ARBs. Biological treatments are less adopted but are vastly studied and have shown potential in controlling the prevalence of ARGs and ARBs and thus need to be explored.

## **Conclusion**

In general, we reviewed 30 research articles to understand the several types of ARGs that are often investigated or identified in various ecosystems to learn about their sources, effects, and current technology for removing these ARGs from the environment. Antibiotics such as chloramphenicols (CPs), tetracyclines (TCs), fluoroquinolones (FQs), sulfonamides (SAs), and macrolides (MLs) were commonly focused in many studies. Antibiotic resistance genes thrived in various aquatic ecosystems, which offered an ideal environment for their collection and replication. Plastic debris has acted as a reservoir for antibiotic resistance genes and a potential vector for antibiotic-resistant bacteria. At the same time, WWTPs have been identified as potential hubs for spreading antibiotic resistance determinants into aquatic ecosystems. Technological advancement in WWTPs is important to eliminate ARGs more efficiently, while some less technology-reliance methods should be continuously studied and adopted. In conclusion, ARGs have found their way to either directly or indirectly impact aquatic ecosystems and other living organisms. It is up to us how to control the abundance of ARGs or learn to adapt to the current conditions.

## **Acknowledgments**

All authors would like to acknowledge the team of lecturers involved in the design and teaching of the subject code BIO330 that make this paper possible to be published in its form.



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