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Complex migration of antibiotic resistance in natural aquatic environments☆

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ABSTRACT

Antibiotic resistance is a worsening global concern, and the environmental behaviors and migration patterns of antibiotic resistance genes (ARGs) have attracted considerable interest. Understanding the long-range transport of ARG pollution is crucial. In this study, we characterized the dynamics of ARG changes after their release into aquatic environments and demonstrated the importance of traditional chemical contaminants in the transmission mechanisms of ARGs. We hypothesized that the main route of ARG proliferation switches from active transmission to passive transmission. This antibiotic-dominated switch is motivated and affected by non-corresponding contaminants. The effect of anthropogenic activities gradually weakens from inland aquatic environments to ocean environments; however, the effect of changes in environmental conditions is enhanced along this gradient. The insights discussed in this study will help to improve the understanding of the distribution and migration of ARG pollution in various aquatic environments, and provide a modern perspective to reveal the effect of corresponding contaminants and non-corresponding contaminants in the process of antibiotic resistance proliferation.

1. Introduction

A large number of studies have shown that the misuse and abuse of antibiotics has accelerated the emergence of drug resistance (Wollenberger et al., 2000; Hu et al., 2008; Rysz and Alvarez, 2004). The emergence and diffusion of antibiotic resistance within a wide range of infectious agents is a growing concern for global public health. In particular, the proliferation of antibiotic resistance genes (ARGs) and antibiotic resistance bacteria (ARB) will increase the migration, transformation and diffusion of antibiotic resistance among environmental bacterial groups, eventually leading to environmental bacteria becoming a natural reservoir for the various ARGs. Gene pollutants have the biological characteristics that "may be reproduced or transmitted" combined with the physicochemical characteristic of "environmental persistence," resulting in ecological damage that is more direct than ordinary chemical pollutants, more easily spread, and more difficult to control and eliminate.

In recent years, research into the persistence of antibiotic resistance in the environment has increased. ARGs and ARB have been isolated from the soil, sediment, urban sewage, aquaculture wastewater, rivers and even the marine and polar environments (Zhang et al., 2016; Yang et al., 2017; Joy et al., 2014; Calero-Cáceres et al., 2017; Na et al., 2014). These results were not only shown in developing countries with a larger use of antibiotics and a wider range of use but also in developed countries that have relatively stricter usages. The antibiotic usage rates in inpatient and surgical operations in China were 80%, and 95%, respectively, which were both far higher than in developed countries such as the Europe and the United States which exhibited rates of 22%–25%, as well as the international average of 30% according to the World Health Organization (Zhang, 2012).

ARG pollution is released from its source pollution and can enter the natural aquatic environment both directly and indirectly. Important release sources include wastewaters from hospitals and agricultural livestock systems. For example, Szekeres et al. (2017) found that *sul1* and *sul2*, which are related to sulfonamide, were

present in the hospital wastewater of a Romanian study site. Further, studies have indicated that the aforementioned ARGs were present in the hospital wastewater of a Chinese study site, agricultural wastewater in Italy and swine wastewater in the United States (Li et al., 2016a; Luprano et al., 2016; Zhang et al., 2013). Antibiotic resistance contaminants can be directly released to the primary reception system, which is connected to the release source (e.g., via wastewater treatment plants [WWTPs]) (Szczepanowski et al., 2004; Schlüter et al., 2007). Furthermore, antibiotic resistance contaminants can leach to groundwater or be carried by runoff and erosion to the secondary reception system (groundwater and surface water; Tennstedt et al., 2005; Chen et al., 2007; Auerbach et al., 2007), and finally converge to the tertiary reception system (estuaries and nearby coastal and ocean systems) under the influence of hydrological dynamics. These release and reception systems ultimately lead to complex migration patterns of antibiotic resistance.

Environmental reception systems at all levels run through the entire process of long-distance migration and diffusion of antibiotic resistance (from source to surface water to the ocean) (Fig. 4). The effect of anthropogenic activities becomes gradually weaker, whereas the effect of changes in environmental conditions (pollutants and the physical and chemical factors) is enhanced from the river to the ocean. Different environmental conditions affect the rate of contribution of each mode of transmission in the process of ARG migration, thereby affecting the characteristics of ARG pollution in the environmental medium. This review presents an overview of the studies on antibiotic resistance in different reception systems to reveal the large-scale diffusion of antibiotic resistance and subsequently study its globalization trend. Furthermore, we hypothesized that the main pathway of ARGs proliferation switches from active transmission, which is dominated by corresponding contaminants (antibiotics), to passive transmission, which is motivated and affected by non-corresponding contaminants (heavy metals, organic pollutants and physical and chemical factors). Overall, the insights discussed in this review will help improve the understanding of the distribution and fate of ARG pollution in various aquatic environments, and provide a modern perspective on the effect of corresponding contaminants and non-corresponding contaminants in the process of ARG proliferation.

2. Pollution levels of antibiotic resistance during their migration in various aquatic environments

Since Pruden et al. (2006) put forward ARGs as a new type of environmental pollutant, they have attracted considerable attention in environmental research. Studies mainly in European countries, the US, and China have found multiple varieties of ARGs in wastewater, lakes, rivers, groundwater, and even coastal water (Joy et al., 2014; Yang et al., 2017; Calero-Cáceres et al., 2017; Na et al., 2014). ARGs are not only leached to groundwater or carried by runoff or erosion like typical chemical pollutants, but they can also be vertically transmitted to microbial offspring via proliferation dynamics, or passed among bacteria via horizontal transfer. These various complex transport processes have led to the complicated problem of assessing ARG pollution in the environment.

2.1. Pollution levels about the release source

Applications of antibiotics in medical and aquaculture industries have accelerated the generation and discharge of ARB, eventually resulting in hospital and animal husbandry wastewaters becoming the main sources of ARG distribution into the environment. According to CHINET which surveys bacterial resistance in China (2015) statistics, among 17,309 clinically collected isolates of *Escherichia coli* from 20 Chinese hospitals, the prevalence of ampicillin resistance was as high as 85.5%. In addition, ciprofloxacin and sulfamethoxazole resistant strains accounted for an average of 57.8% and 58.1% of *E. coli*, respectively (Hu et al., 2015). With the increase in resistance levels, at least 700,000 people die due to antibiotic-resistant infections each year, and this figure is expected to exceed 10,000,000 in 2050 (Antimicrobial resistance global report on surveillance, 2014).

In recent decades, researchers have detected 133 different ARGs in hospital, and animal husbandry wastewaters with activity towards 12 types of widely used antibiotics (Fig. 1, Table S1). Among all classes of ARGs detected in different hospital wastewaters, tetracycline resistance genes (*tet* genes) and sulfonamide resistance genes (*sul* genes) have the highest detection frequency. The average detection rate of both ARGs reached 100% (Fig. 1, Table S4). Furthermore, other ARGs also detected frequently include aminoglycoside resistance genes. The concentration and abundance of all different types of ARGs were 10^1–1.81×10^{11} copies/mL and 6.30×10^{-7}–2.81×10^{-1} copies/16S rRNA, respectively, in hospital wastewaters (Fig. 2). Researchers have mainly focused on *Enterococcus* of gram-positive bacteria and *P. aeruginosa* of gram-negative bacteria among the ARB in hospital wastewaters (Varela et al., 2013; Hocquet et al., 2016). For ARB, the resistance to gentamicin and meropenem has received the most attention and the mean resistance rates were 24.1% and 11.8%, respectively (Table S5). Among all classes of ARGs detected in different animal husbandry wastewaters, *sul* genes and *tet* genes have the highest detection frequency with an average detection rate reaching 100% and 99.7%, respectively (Fig. 1, Table S4). The concentration and abundance of all kinds of ARGs were $10^{0.94}$–2.43×10^{11} copies/mL and 10^{-8}–6.30×10^{-1} copies/16S rRNA, respectively (Figs. 2 and 3). For ARB in the animal husbandry wastewaters, researchers have paid more attention to sulfamethoxazole resistance, for which the mean resistance rate is 46.4% (Table S5). This may be related to the extensive use of sulfamethoxazole in animal husbandry. *Bacillus*, *Acinetobacter* and *Vibrio* spp. were most common among the sulfamethoxazole resistant bacteria identified in animal husbandry (Gao et al., 2012a; Hoa et al., 2011).

Overall, the concentration and abundance of all kinds of ARGs is mainly focused on 10^6–10^9 copies/mL and $10^{-3.1}$–$10^{-1.6}$ copies/16S rRNA in the hospital and animal husbandry wastewaters, the median values were $10^{7.1}$ copies/mL and $10^{-2.2}$ copies/16S rRNA, respectively. However, in some severely polluted areas, the concentration and abundance of ARGs reached 10^{10}–10^{11} copies/mL and 10^{-2}–10^{-1} copies/16S rRNA. Many researchers have indicated that the construction and abundance of antibiotic resistance is closely related to the usage of antibiotics in medical and aquaculture industries. For example, Peak et al. (2007) analyzed the correlation between ARGs and antibiotics in cattle wastewater of Kansas and found a strong correlation (r = 0.67, p < 0.01). Chen et al. (2013) also found that the abundance of ARGs had a significant correlation with the concentration of antibiotics in the aquaculture areas of the Pearl River Estuary (winter, r = 0.734, p < 0.0001; summer, r = 0.740, p < 0.0001). Kerry et al. (1996) compared aquaculture ponds to a river without antibiotic pollution and found that the number of bacteria with tetracycline and chloramphenicol resistance was significantly higher in the aquaculture ponds. This may explain why the main type of antibiotic resistance is different from different release sources. The types of ARGs in the hospital wastewaters mainly correspond with the antibiotics used in human applications, such as tetracycline and aminoglycoside, but the types of ARGs in the animal husbandry wastewaters mainly correspond with the antibiotics used in animals, such as sulfonamides and tetracycline.