Research Progress of Antibiotic Resistance Genes (Args) Pollution in Drinking Water Source

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Abstract. In recent years, the problem of the pollution of antibiotic resistance genes (ARGs) in water environment is becoming more and more serious. As a new environmental pollutant, people have been paid more attention to the harmfulness of ARGs in drinking water sources. The source of ARGs, pollution status, detection and identification technology and water treatment technology have been systematically reviewed, and the future research directions of ARGs were prospected.

1. Introduction
Antibiotics are widely used in medical treatment, animal husbandry, animal husbandry and other fields, making important contributions to the development of human society. However, the long-term abuse of antibiotics not only causes environmental pollution, but also induces the generation of antibiotic resistance genes (ARGs) [1]. After the use of antibiotics by humans and animals, most of the antibiotics that are not fully absorbed and utilized are discharged from the body with excreta, and enter rivers, lakes and groundwater through surface runoff, rainwater washing and soil [2]. When microorganisms in water are contaminated, their potential resistance is expressed by horizontal gene transfer or mutation [3]. Once microorganisms have some ARGs, the movable genetic factors and integrons in their genomes can spread and diffuse through transformation, transduction, conjugation, etc [4]. Moreover, pollutants such as heavy metals and detergents in the environment can promote the horizontal transfer of ARGs to some extent [5]. ARGs have the characteristics of "replicability or transmission" and "not easy to die out or environmental persistence". They can be persistent residues, migration and transmission in the environment. Therefore, ARGs are often more harmful to water and human health than antibiotics themselves [6]. At present, many researchers have detected many kinds of ARGs, including tetracyclines, macrolides and sulfonamides, in water sources, drinking water treatment processes and even in pipeline water [7]. In China, antibiotics are heavily used about 210,000 tons of antibiotics every year, of which more than 50,000 tons are discharged into the water environment [8]. More than 80,000 people die each year from antibiotic resistance in China, according to a new WHO study. If the current situation is not improved, it is expected that 1 million people will die in China by 2050. These events and data make people gradually realize that ARGs have seriously threatened human health and ecological security.
Aiming at the problem of ARGs pollution in drinking water sources, studies on ARGs pollution status as well as their detection technology, water treatment technology are summarized, and suggestions and prospects for future research focus and direction are put forward in this paper.

2. Current status of antibiotic resistance gene contamination in drinking water sources

2.1. River Water

The total concentration of antibiotics in rivers of China is relatively high, which is influenced by population density and distribution factors of pharmaceutical, agriculture and aquaculture industries.

The density of antibiotic emissions in eastern China is more than 6 times higher than that in Western China [9]. The survey shows that the highest concentration of antibiotics in rivers in China can reach 7560 ng/L, with an average of 303 ng/L, while in the United States 120 ng/L, Germany 20 ng/L and Italy only 9 ng/L are far lower than those in China [10]. Contamination of ARGs caused by abuse of antibiotics is becoming more and more serious. Yang et al. investigated 8 ARGs in Haihe River of Tianjin, sulfonamide ARGs sul1 has the highest detection rate and the maximum abundance can reach $6.4 \times 10^5$ copies/mL [11]. 9 ARGs were detected by Ling et al. in the southern Beijiang River. Tetracycline resistance gene tetC content was the highest, ranging from $8.30 \times 10^2$ to $1.32 \times 10^3$ copies/16SrDNA [12]. Xu et al. found 2 sulfonamide ARGs, 6 tetracycline ARGs and 2 integron genes in seven rivers in Beijing. Sul 1, tetA and tetE were the most abundant genes in the rivers, with the highest abundance of $2.3 \times 10^9$ copy/mL [3]. Jiang et al. Detected 2 sulfonamide ARGs, 8 tetracycline ARGs and 1 lactam ARG in Huangpu River by PCR Technology, the content of these genes ranged from $3.66 \times 10^1$ to $1.62 \times 10^5$ copy/mL [13]. Stoll et al. detected more than 20 kinds of ARGs in Rhine River, Germany. Among them, the detection rate of sulfonamide resistance gene was 77%~100%, and that of macrophage resistance gene ermB was 68%, which were higher than 18% in surface water of Australia [14]. The above research shows that river water has become the main gene reservoir of ARGs in the environment, and is closely related to the type and intensity of human activities.

2.2. Lake Reservoir Water

Lakes and reservoirs have many functions, such as drinking water source, sightseeing, irrigation and so on. They are vulnerable to human activities and are more conducive to the accumulation and dissemination of ARGs. Yang et al. found that 15 representative lakes in the middle and lower reaches of the Yangtze River were polluted by ARGs to vary degrees. Sulfonamide and tetracycline ARGs were widely distributed. The average relative abundance of tetG ARGs was the highest, which was $4.74 \times 10^{-3}$ copies/16SrRNA [15]. The contents of ARGs in Shahu, Nanhu and Ziyang lakes in the central city of Wuhan are also high. The content of sulfonamide ARGs is between $10^{-4}$ and $10^2$ copies/16SrRNA, and the abundance of tetracycline ARGs is between $10^{-5}$ and $10^5$ copies/16SrRNA. Human activities and eutrophication seriously affect the distribution of ARGs in lakes [16]. Zhang et al. studied the water quality indicators of water supply reservoirs in Beijing, Tianjin and Hebei areas in China which all met the Class II of "Surface Water Environmental Quality Standard" (GB 3838-2002). It was found that the resistance gene abundance detected in Panjiakou and Guanting reservoirs were the highest. The detection rates of sul1, tetM and ermB were 100%, and the abundance ranged from $10^6$ to $10^8$ copy/mL, and tetM is closely related to COD and Chl-α [17]. Thus, even if the conventional physical, chemical and biological indicators meet the relevant standards, new pollutants such as ARGs may have a higher detection rate and abundance, and bring potential risks to aquatic ecosystems and human health. Many investigations have found that many lakes and reservoirs abroad are also polluted by ARGs. Czekalski et al. found that tetracyclines, sulfonamides and macrolides ARGs were detected in Lake Geneva, Switzerland [18]. Graham et al. detected 3 β-lactamase ARGs, 2 erythromycin ARGs and 5 tetracycline ARGs in a reservoir in Cuba [19]. ARGs were found in 24 large urban water supply reservoirs in the United States, affecting at least 40 million people. Xiet al. not only found that the reservoirs in Michigan and Ohio contain ARGs, but also found that the ARGs content in the pipeline water is higher than that
in the outlet water and the water supply reservoir [20]. The above results show that the water bodies of lakes and reservoirs around the world have been threatened by ARGs to vary degrees.

3. Detection of antibiotic ARGs

3.1. PCR Technology
Polymerase chain reaction (PCR) was invented in 1985 by Kary Banks Mullis, a scientist from PE-Cetus Company in the United States, a molecular biology technique for amplifying and amplifying specific DNA fragments in vitro. Its basic principle is similar to the natural replication process of DNA. In 2-3 hours, the amplification of the target gene can be millions of times [21, 22]. When detecting ARGs by PCR technology, only DNA extraction of target genes is needed, and no microorganisms need to be isolated and cultured in advance, which reduces the interference of experimental steps and environmental factors. However, DNA polymerase will be inactivated at high temperature, so new DNA polymerase will be added to each cycle, which makes the experiment complicated and expensive. This is also a major problem that restricts the application of PCR technology. Leclercq et al. used PCR technology to detect macrolide ermB and mefA, tetracycline tetM and other ARGs in the lower reaches of a river in the United States [21]. Zhang et al. detected 4 tetracycline ARGs, 2 sulfonamide ARGs and 1 integron gene in samples of sewage treatment plants in Zhejiang area by using PCR technology, and the detection rate all 100% [22]. However, in practical application, PCR technology is often affected by many factors, such as the appearance of amplification reaction inhibitors, random errors in nucleic acid extraction and contamination of amplification products, which lead to false negative and false positive results, and can not accurately quantify the ARGs. Therefore, repeated measurements are needed to ensure the accuracy of the final results.

3.2. Realtime fluorescence quantitative PCR
Realtime fluorescence quantitative PCR (RTFQ PCR) refers to the real-time monitoring of the process of PCR by adding fluorescent groups as fluorescent signals in the process of PCR amplification. When the fluorescent group binds to the amplified product, the fluorescence intensity stimulated is proportional to the amount of the amplified product, so that the resistance gene can be quantitatively detected accurately. Because of its high efficiency, specificity, accuracy and observability, fluorescence quantitative PCR technology has been widely used in practice. Especially real-time, it can observe the whole PCR process at any time, and the result is fluorescence display, which is easier to observe. But the disadvantage is sometimes false positive, which affects the final experimental results. Duan et al. used RTFQ PCR to detect 6 ARGs in the influent of four sewage treatment plants in Harbin, the detection rate of 6 ARGs was 100%, and there were some differences in the abundance of these 6 ARGs in different sewage treatment plants [23].

3.3. High-throughput sequencing technology
High-throughput sequencing technology is a revolutionary progress in the development of gene sequencing technology. It can sequence millions of DNA molecules at the same time, and achieve in-depth, detailed and comprehensive analysis of species' transcripts and genomes. Therefore, it is also known as the“Next-generation”sequencing technology [24]. The advantages of high throughput sequencing technology compared with traditional sequencing methods are as follows: firstly, using chips can simultaneously sequence millions of points, also known as large-scale parallel sequencing; secondly, no electrophoresis is needed, which is easy to operate and saves cost and time. Based on the above advantages, high throughput sequencing technology is not only widely used in gene sequencing, but also applied in functional genomics research. Jiang et al. used high-throughput sequencing technology to study the lower reaches of the Yangtze River. 118 kinds of ARGs were detected in reservoir water and 124 kinds of ARGs were detected in sediments. It was found that there was a significant correlation between the integrons and the abundance of ARGs in water samples [25]. High throughput sequencing technology also has some shortcomings in the application process. Although the speed of sequencing
has been improved, it is difficult to analyze the large amount of data and not suitable for small-scale sequencing. Overall, in the future, with the continuous improvement of microbial species database and sequencing technology, high throughput sequencing technology will become one of the important means of resistance gene detection. (Table 1)

| Detection technology                      | advantage                                      | defect                          | reference       |
|-------------------------------------------|-----------------------------------------------|---------------------------------|-----------------|
| PCR                                       | Wide application field, few interference factors | Low accuracy, repetitive detection | [21] [22]       |
| RTFQ PCR                                  | Real-time monitoring, easy observation, strong specificity, high accuracy | Occasionally false positive results occur | [23]            |
| High-throughput sequencing technology      | Suitable for large-scale sequencing, easy operation, cost saving and time saving | Large amount of data, difficult to analyze | [24] [25]       |

4. Removal of antibiotic ARGs by water treatment process

4.1. Conventional treatment process

At present, more and more ARGs are detected in drinking water sources. It is found that different drinking water treatment processes have different removal effects on different ARGs. The content of some kinds of ARGs even increases after treatment. Coagulation and precipitation are the key stages of resistance gene removal. After coagulation and precipitation treatment, the ARGs content of raw water decreases to a great extent. Coagulant can combine suspended substances and organic substances with free antibiotic resistant bacteria (ARB) to form macromolecular polymers. After precipitation treatment, antibiotic resistant bacteria in water are reduced, and the resistance gene fragments are removed, thus reducing the relative abundance of ARGs in treated water samples. Relevant data show that the average removal rates of ARGs in water by coagulation process of two drinking water plants in Yangtze River Delta basin are 74.71% and 56.37%, and the average removal rates of ARGs in raw water of Zhejiang drinking water plant are 84.23% after coagulation and sedimentation treatment [26]. Wang et al. found that after coagulation treatment, the content of ermB gene decreased from $7.02 \times 10^4$ copy/mL to $5.24 \times 10^3$ copy/mL [27]. Zhang et al found that when PACl (polyaluminium chloride) and PFS (polyferric sulfate) were used as coagulants and the dosages were 0.85 and 0.50 mmol/L respectively, coagulation precipitation-UF process could remove all kinds of ARGs in water by 2-3 orders of magnitude, of which PACl-UF process had the best effect, with removal rates of 2.51-3.52 orders of magnitude [28].

Disinfection process can inhibit the expression of related transfer genes by reducing the survival rate of donor bacteria, thus removing part of ARGs in water. For example, Lin et al. found that lower residual chlorine inhibited the expression of genes related to extramembrane protein gene and DNA transfer, and reduced ARGs abundance by reducing gene transfer rate [29]. The content of tetG decreased from $7.94 \times 10^5$ copy/mL to $1.63 \times 10^5$ copy/mL after disinfection in drinking water plants in the lower reaches of Jiulong River [27]. Xiet al. found that sulfonamide ARGs decreased significantly after chlorination in water plants in Michigan and Ohio [20]. However, some studies have found that disinfection process has selective enrichment effect on ARGs. Shi et al. found that the content of emPC, ermA, ermB and other 7 ARGs increased after chlorination disinfection. It can be seen that although the disinfection process can remove some types of ARGs, it may also play a role in promoting the horizontal transfer of some ARGs [30].

4.2. Advanced treatment process

Ozone-biological activated carbon process combines ozone oxidation, ozone disinfection, adsorption of activated carbon and microbial degradation, which has obvious removal effect on antibiotics and other organic matters. Relevant studies have shown that ozone can rapidly eliminate Lincomycin molecules in one hour; J. Rivera-Utrilla et al. found that after the contact of sulfonylamidazole with ozone in water
into the activated carbon column, the content of sulfonylamidazole decreased significantly, thereby reducing the induction of ARGs [31].

Membrane separation technology uses pressure as the driving force, uses mechanical sieve filtration to separate impurities in water, and ensures the stability and reliability of water quality. Wang et al. studied the effect of nanofiltration and reverse osmosis membranes on the removal of tetracycline ARGs in water. It was found that nanofiltration and reverse osmosis membranes not only have strong adsorption capacity for tetracycline antibiotics, but also have good removal efficiency for tetracycline ARGs [32]. Böckelmann et al. considered that ultrafiltration membrane process also had certain effect on the removal of some kinds of ARGs. Studies showed that the content of sulfonamide and tetracycline ARGs in water treated by ultrafiltration membrane process decreased, but the content of other ARGs increased [33]. At present, the existing water treatment process can only remove some kinds of antibiotics and ARGs in water, but it can not be completely eliminated. Therefore, it is necessary to continuously improve the water treatment process in order to remove the antibiotics and ARGs in the water to the greatest extent, to a certain extent, to curb the spread and spread of ARGs, so as to reduce its harm to human body and water pollution.

5. conclusions and Prospects

Our country is a big country in the production and use of antibiotics. There is widespread abuse of antibiotics in medical industry and animal husbandry. The abundance of ARGs detected in drinking water sources is increasing. In addition, water treatment technology can not effectively remove ARGs, which poses certain risks to human health and the environment. Therefore, we should strengthen people's awareness of reducing the use of antibiotics and the spread of ARGs in the environment. At the same time, we should vigorously carry out the following research:

1) To study the sources, distribution and transmission of ARGs in water and their environmental risks, establish an assessment system and risk early warning mechanism of ARGs ecological environment safety, and raise people's awareness of ARGs pollution prevention.

2) Standardize ARGs detection technology and methods, understand the status of ARGs pollution in various drinking water sources, and reduce its harm to human health.

3) Accelerating the research and improvement of ARGs removal technology in water treatment process, exploring more efficient and practical resistance gene control and treatment technology by using the advantages of combination process.

Acknowledgements

This work was jointly supported by the Shandong Province National Science Foundation, China (ZR2017MC047), the Critical Patented Projects in the Control and Management of National Polluted Water Bodies (2017ZX07502003-06), the Special Project of Taishan Scholar Construction Engineering (ts201712084), and the Science and Technology Project of Jinan Urban and Rural Water Bureau “Studies on rural non-point source pollution prevention and control technology in Jinxuichuan watershed, Jinan” (2017266).

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