# Study on the Characteristics of Antibiotic Resistance Genes in Different Biological Treatment Systems

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**Abstract.** Due to the irrational use of antibiotics, antibiotic resistance genes are widely present in the environmental media of our lives.Antibiotics have potential environmental and public health risks, and they bring harm to the environment in which we live.Sewage treatment plants are antibiotic resistance genes' repository and important process for removing antibiotic resistance genes. The different processes they use in sewage treatment plants, the effect of removing antibiotic resistance genes is also different. In order to discuss the impact of different processes on the removal of antibiotic resistance genes, we selected three wastewater treatment plant samples with different treatment processes for testing, and used the fluorescence quantitative analysis technology of 16SrRNA gene to study the abundance, distribution and diversity of antibiotic resistance genes in different treatment processes.The results showed that the AAO process, the oxidation ditch process, and the CASS process all have high removal effect on antibiotic resistance genes, they all can reduce the diversity and abundance of antibiotic resistance genes, and the oxidation ditch process is the best process of the three treatment processes.

**Keywords.** Antibiotic resistance, antibiotic resistance genes, 16SrRNA, wastewater treatment plant, treatment process

## 1. Introduction

Antibiotics are broadly used for the control of human and animal diseases, Therefore, medicine, animal husbandry and aquaculture are the main places where antibiotic resistance genes are produced. Some unreasonable abuse can make bacteria develop antibiotic resistance. Antibiotics will spread and accumulate in a variety of ways, thereby endangering human life and environment. In addition to the chemical contamination of antibiotics themselves, they can also accelerate the production of antibiotic resistance genes and resistant bacteria. The resistance genes and resistant bacteria will pose threat to the health of humans and animals [1].

Wastewater treatment plants mainly receive domestic sewage, including hospital wastewater, industrial wastewater and other wastewater. The bacteria from wastewater is the main source of microorganisms in wastewater treatment plants. Although the wastewater treatment plant is considered to be the main site for the reduction of

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resistant bacteria and resistant genes, but the studies have shown that after passing through the various treatment units of the wastewater plant, the effluent and sludge still contain a considerable concentration of resistant bacteria and resistance genes. They will enter the environment and pose a threat to human health and safety [2-3].

In this study, we collected 3 samples from Lanzhou Wastewater Treatment Plant: Lanzhou Yan'erwan Wastewater Treatment Plant (Y), Lanzhou Heping Wastewater Treatment Plant (H), Lanzhou Yanchangbao Wastewater Treatment Plant (B), using 16SrRNA gene Fluorescence quantitative analysis technology to study the abundance, distribution and diversity of antibiotic resistance genes in samples.

## 2. Materials and Methods

### 2.1. Sample Collection and Pretreatment

In preparation for the experiment, we collected wastewater and sludge samples from three wastewater treatment plants in Lanzhou, including influent, activated sludge and effluent. Lanzhou Yan'erwan Wastewater Treatment Plant(Y) uses AAO process, Lanzhou Heping Wastewater Treatment Plant(H) uses oxidation ditch process, and Lanzhou Yanchangbao Wastewater Treatment Plant(B) uses CASS process. In order to ensure a better calculation of the resistance gene removal effect, when collecting samples, we ensure that the influent water and the treated effluent are the same batch, First, add ethanol for pretreatment, then put the collected samples to a low-temperature sampling box and quickly transport back to the laboratory for storage in a refrigerator at -20°C for DNA extraction from environmental samples.

### 2.2. Extraction and Identification of sample DNA

The collected samples are extracted with Ezup column soil genomic DNA extraction kit. The kit uses Buffer SCL to lyse the sample to release genomic DNA, and then uses Buffer SP and chloroform to remove impurities such as proteins. Buffer SCL can remove humic acid from the soil and avoid the interference of humic acid on subsequent experiments. Using this kit, 5-10μg DNA can be obtained from 200mg of soil. Weigh 100-300 mg of soil, add 400 μL of 65°C preheated Buffer SCL, and perform extraction according to the instructions in the subsequent steps. Finally, the obtained DNA solution was stored at -20°C or used directly for subsequent experiments.

### 2.3. Fluorescence Quantitative Analysis Technology of 16SrRNA Gene

Roche480 was used to determine the 16SrRNA genes of all the bacteria samples. The quantitative primers were carried out in a 20μL reaction system. The amplified fragments of the 16SrRNA gene and the vector pMD19-T were ligated and transformed into E. coli competent cells, and the plasmids were extracted by activation and culture to prepare fluorescence. The standard plasmid needed to quantify the standard curve. The qPCR reactions of all samples were subjected to 3 technical replicates, including negative controls [4].

## 2.4. Data Analysis

Gene relative copy number refer to the method of Looft et al, using formula (1) to estimate, and formula (2) for transformation can calculate the absolute abundance of antibiotic resistance genes.

$$\text{Relative copy number of gene} = 10^{(31-Ct)/(10/3)} \qquad (1)$$

Absolute Abundance of Resistance Gene(copies/L)=Relative copy number of resistance gene÷Relative copy number of 16SrRNA gene×Absolute copy number of 16SrRNA gene [5]                                                                                      (2)

## 3. Results

### 3.1. Diversity of Resistance Genes in Sewage Treatment Plants

There are 117, 128, 97 antibiotic resistance genes were detected in the samples of Lanzhou yan'erwan wastewater treatment plant (Y), Lanzhou Heping wastewater treatment plant (H), Lanzhou yanchangbao wastewater treatment plant (B), respectively (figure 1a). Depending on the types of antibiotics, antibiotic resistance genes could be classified into eight categories: Aminoglycoside, Beta_Lactamase, MLSB (macrolide-lincosamide-streptogramin B), Tetracycline, Sulfonamide, Multidrug, Vancomycin and Others.

According to the detection, these wastewater treatment plants are similar in the composition of the resistance genes. Types of antibiotics resistance genes were relatively high, which include previously described all categories of antibiotic resistance genes. However, the species number of Sulfonamides resistance genes and Vancomycin resistance genes were less. Sulfonamides are broad-spectrum antibiotics with low price and stable properties. They are widely used in aquaculture [6-7]. Vancomycin is considered as the last line of defense for the treatment of critically ill patients [8]. The emergence and distribution of Sulfonamides and Vancomycin resistance genes suggests that corresponding types of antibiotics were likely exist in wastewater treatment plants, which had potential environmental and public health risks [9]. In addition, the number of antibiotic resistance genes detected in Yanchangbao Wastewater Treatment Plant were less than that of Yan'erwan Sewage Treatment Plant and Heping Sewage Treatment Plant. According to the resistance mechanism, antibiotic resistance genes could be divided into four categories: deactivation, efflux, protection and unknown [10]. Among multiple mechanisms for process, we considered the deactivation as the most dominant mechanism of antibiotic resistance in wastewater treatment plants, accounting for 49.77% of cases. Followed by efflux and protection, accounted for 27.91% and 16.74% respectively (figure 1b).
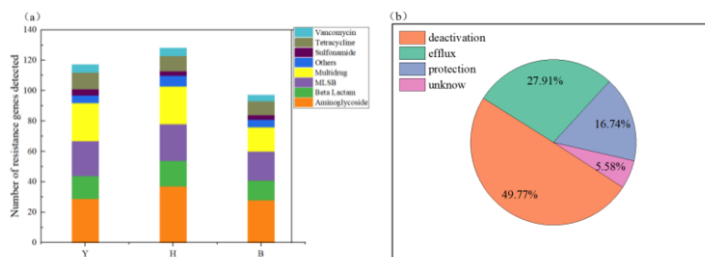
**Figure 1.** (a) Types of antibiotic resistance genes; (b) Relative proportion of resistance mechanisms.

## 3.2. Abundance of Resistance Genes In Wastewater Treatment Plants

A total of 212 resistance genes and 42 MGEs were detected in 56 samples. Among them, 209 ARGs were detected in influent samples, 184 ARGs were detected in activated sludge, and 126 ARGs were detected in effluent. According to the test results that the sewage treatment process has significantly reduced the diversity of resistance genes, Among the three environmental samples, resistance genes in influent samples has the highest diversity. Aminoglycoside, Multidrug, Sulfonamide, MLSB and Tetracycline are the main ARG species, occupying 89%~93% of the abundance of resistance genes. The sewage treatment process can significantly decreased the abundance of ARGs and removed about 1 to 2 orders of magnitude resistance genes. And the most abundant resistance gene was detected in activated sludge (figure 2a).

In three sewage treatment plants, eight resistance genes and one MGEs were detected in all samples. The main ARGs that all existed in the three environment are Aminoglycoside (aacC4, aac(3)-Via and APH(6)-Ia), Beta_Lactamase(blaOXY-1), Multidrug (sugE and czcA),  MLSB(erm(35)), Tetracycline(tetA(P)). In addition, the mobile genetic element MGE (IS1247) also persisted after treatment. They accounted for the relative abundance of the detected resistance genes in influent, activated sludge and effluent, respectively 17.4%, 23.6% and 83.8% ratio (figure 2b).
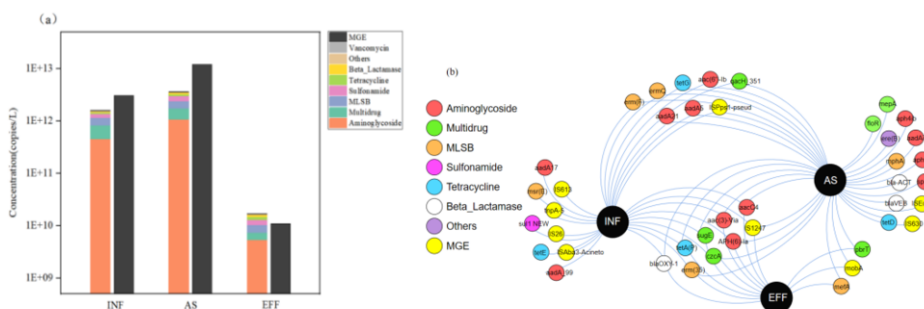


**Figure 2.** (a) Antibiotic resistance gene abundance map; (b) Distribution map of antibiotic resistance genes in three environments.

### 3.3. Comparison of Treatment Processes in Different Sewage Treatment Plants for Removal of Resistance Genes

Figure 3 shows the absolute abundance of resistance genes in samples tested by different sewage treatment plants. The absolute abundance of antibiotic resistance genes in the influent samples of the three sewage treatment plants can reach $10^4 \sim 10^8$ copies$\cdot$g$^{-1}$, In general, the Yan'erwan Wastewater Treatment Plant (Y) is 3-4 orders of magnitude lower than the samples from the other two wastewater treatment plants. Among the influent samples, Aminoglycoside antibiotics in the Yan'erwan Wastewater Treatment Plant (Y) showed the highest abundance ($3.9 \times 10^5$ copies$\cdot$g$^{-1}$), Vancomycin resistance genes show the lowest abundance (0). In the effluent samples, the AAO process has a significant effect in removing antibiotic resistance genes, and the number of resistance genes is reduced by 10-11 orders of magnitude. Among them, the removal of Aminoglycosides, MLSB antibiotics, and other types of antibiotics with efflux pump effect are better. Among the influent samples of Heping Sewage Treatment Plant (H), MLSB antibiotics showed the highest abundance ($1.9 \times 10^7$ copies•g$^{-1}$), Vancomycin resistance gene is the same as Y plant, showing the lowest abundance (0). In the effluent of Plant H, the eight types of resistance genes get well removed, and the oxidation ditch process was effective in removing sulfonamide resistance genes. Yanchangbao Sewage Treatment Plant (B) uses CASS technology to remove resistance genes, Among influent samples, Aminoglycoside resistance genes showed the highest abundance ($1.5 \times 10^8$ copies•g$^{-1}$), Vancomycin resistance genes show the lowest abundance ($5 \times 10^4$ copies•g$^{-1}$). As is shown in figure 3, Vancomycin resistance genes of Plant B were all removed in influent, and the abundance of other resistance genes are decreased in different degrees. Comprehensive analysis of the three sewage treatment plants, in the influent samples, the abundances of Heping Sewage Treatment Plant (H), Yanchangbao Sewage Treatment Plant (B), and Yan'erwan Sewage Treatment Plant (Y) show decreasing trend. After the removal of different sewage treatment processes, the effluent samples showed a decreasing trend in the abundance of Yanchangbao Sewage Treatment Plant (B), Yan'erwan Sewage Treatment Plant (Y), and Heping Sewage Treatment Plant (H). Comprehensive comparison of the three processes, the oxidation ditch process has the best effect in removing eight types of resistance genes.
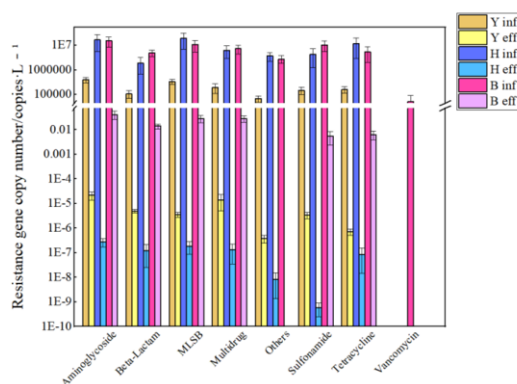


**Figure 3.** Absolute abundance of antibiotic resistance genes.

## 4. Discussion

(1) There are many types of antibiotic resistance genes. We have detected eight types of resistance genes from three sewage treatment plants.

(2) According to the resistance mechanism, antibiotic resistance genes could be divided into four categories: deactivation, efflux, protection and unknown.The deactivation accounts for the largest proportion of antibiotic resistance. The second is the efflux pump mechanism and ribosome protection mechanism.

(3) According to the comparison of influent, activated sludge and effluent, wastewater treatment not only reduces the abundance of ARGs, but also removes about 1-2 orders of magnitude of resistance genes. Among them, resistance genes are the most abundant in activated sludge.

(4) Comprehensive comparison of the three processes,  the oxidation ditch process has the best effect in removing eight types of resistance genes.

## Acknowledgement

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