Research Article

Metagenomics Analysis of Microbial Species and Antibiotic Resistance Genes (ARGs) in Untreated Wastewater from Different Types of Hospitals in Hangzhou

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Antibiotic resistance genes (ARGs) are contaminants that can propagate through a variety of environmental media. They contribute to an increase in the number of antibiotic-resistant microbes and thereby pose a danger to human health. Discharge from hospitals is the most significant contributor of ARGs and antibiotic-resistant bacteria to the natural environment. A comprehensive understanding of the microbiological structure and the distribution of ARGs in hospital wastewater can facilitate appropriate treatment of such wastewater and can improve our understanding of the pathophysiology of several epidemic illnesses. In this work, metagenomics techniques were used to compare the microbial species and ARGs in a control group with those in untreated wastewater from three types of hospitals (a general hospital, a hospital of traditional Chinese medicine, and an oral specialty hospital). The microbiota found in the hospital wastewater represented 6,415 species and 244 phyla. The composition of the bacterial community in the wastewater from the three hospitals was significantly different from that in the control group. The ARGs in the samples were also analyzed using the Antibiotic Resistance Genes Database (ARDB) and Comprehensive Antibiotic Resistance Database (CARD). Finally, the link between abundant species and ARGs in the samples was examined. The findings of this study indicate that a connection exists between the microbial species and the ARG composition found in the wastewater samples. A variety of distinct genera of ARGs, each having their own unique correlations, have been found in wastewater from various hospitals. Consequently, the ARGs and microbial species found in the untreated wastewater from various hospitals have unique characteristics. Therefore, more detailed protocols need to be established to treat wastewater from various types of hospitals. Further studies should examine whether a connection exists between the various microbial species found in the wastewater of various types of hospitals and certain illnesses.

1. Introduction

Antibiotic resistance (AR) refers to the ability of some microbial subgroups (usually bacterial species) to survive after exposure to one or more antibiotics [1]. Antibiotic-resistant pathogens are known as antibiotic-resistant microbes. Some microbes naturally possess AR, whereas others acquire AR by obtaining exogenous ARGs through horizontal gene transfer [2]. ARGs can generate antibiotic resistance through several molecular mechanisms. (1) ARGs can substitute antibiotic active groups, change the structure of antibiotics, and inactivate antibiotics [3]. (2) They can modify antibiotic targets to block the combination of antibiotics with the target [4]. (3) They can expel antibiotics from cells via a specific or universal efflux pump [5]. (4) They can secrete bacterial enzymes that break down antibiotics [6]. ARGs can spread to various microbial species and persist in the environment for a long time. In recent years, they have been considered more hazardous than antibiotics. Thus, research on controlling the environmental pollution

caused by ARGs has received increasing attention. Most ARGs are introduced into natural ecosystems by humans and animals, and it is therefore critical to analyze the ARGs in wastewater from different types of hospitals [7].

Wastewater released from hospitals (general hospitals, specialist hospitals, and other types of hospitals) to the natural environment or urban pipes is referred to as hospital wastewater [8]. The quality of hospital wastewater varies according to the nature, size, and location of hospitals. A previous study suggested that the volume of wastewater discharged from each ward bed was approximately 200–1,000 L/d [9]. Pathogens (e.g., parasite eggs, pathogenic bacteria, and viruses) are the most common pollutants in hospital wastewater, followed by organic debris, floating and suspended particles, and radioactive contaminants [10]; these pollutants are highly hazardous to the environment. Some scholars have explored sewage treatment systems in four hospitals in Canada [11]. The results indicated that the discharge amount of 20 drugs has reached $1,456\,g\,a^{-1}$, and the discharge amount of various drugs ranges from 3.07 (mefenamic acid) to $378\,g\,a^{-1}$ (oxazepam). Owing to "selective pressure," it is easier to transfer ARGs between different microbial species in this environment. The results of a previous study on an urban pharmaceutical and chemical wastewater plant demonstrated that the microbes in the wastewater of this plant mainly included the genera Proteobacteria and Hyphomicrobium, and the species *Hyphomicrobium zavarzinii* [12]. A total of 74 types of ARGs were detected, of which sav1866, dfr E, and mfd were the most abundant. Network analysis revealed the coexistence pattern between ARGs and microbial taxa (operational taxonomic units (OTUs)), namely, that ARGs were highly correlated with microbial taxa at the genus level in the wastewater plant.

The types and quantities of antibiotics in hospital wastewater vary significantly between nations and regions. This can be attributed to the considerable differences in therapeutic regimens and antibiotic treatment durations in different hospitals. In a previous study on three hospitals in Luzhou, Sichuan Province, Song et al. [13] sequenced metagenomes in wastewater from three distinct types of hospitals (a general hospital, a stomatology hospital, and a traditional Chinese medicine (TCM) hospital). They found that the drug contents in wastewater varied with the type of hospital, and ARGs were specific to the microbial species in the wastewater. Significant differences have also been noted in the dosages and types of drugs even in different clinical departments in the same hospital and among different doctors in the same department [14]. Antibiotics, such as amoxicillin, cefuroxime, levofloxacin, azithromycin, and metronidazole, are routinely used to treat infections. TCM departments often use natural antibacterial herbs. These variables may contribute to variations in the bacterial population and incidence of ARGs in wastewater from various hospitals [15].
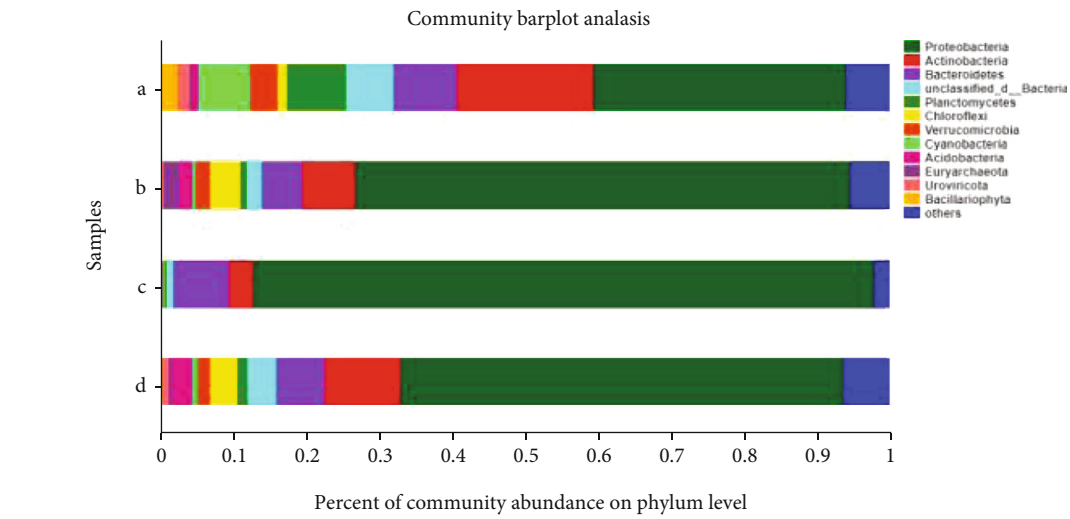
Although the microbes and ARGs in hospital wastewater have been explored previously, the sample sizes have been small, and a control group has been lacking. Moreover, the composition of wastewater may be completely different in different regions and hospitals. Hence, it is necessary to con-duct different studies to ensure favorable environmental protection. Hangzhou, the provincial capital of Zhejiang Province, has a densely distributed water reticulation system, which is essential for the treatment of hospital wastewater. In this study, we evaluated untreated wastewater from three hospitals in Hangzhou, namely, the First Affiliated Hospital, Zhejiang University School of Medicine (FAHZU, Group A), Hangzhou Hospital of Traditional Chinese Medicine (HZTCM, Group B), and Hangzhou Dental Hospital (Group C). Untreated domestic wastewater from the dormitory of Zhejiang University (Group S) was used as the control group for comparison. Initially, we assumed that there would be different therapeutic regimens and medication habits among clinicians in these hospitals and that this would have an impact on the bacterial ecology as well as the distribution of ARGs in wastewater. We further assumed that there may be a link between changes in the bacterial ecology and the distribution of ARGs. These assumptions were validated using metagenomic sequencing technology. The findings of this study are expected to contribute to the formulation of a specific measure for the pretreatment of hospital wastewater before it is discharged into the sewage system and help mitigate the environmental pollution caused by ARGs.
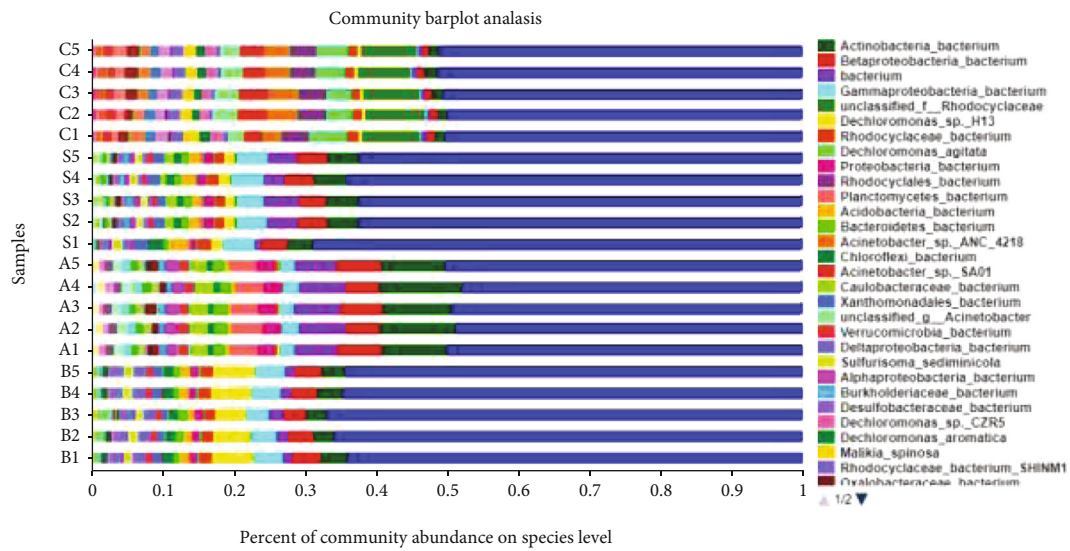
2. Materials and Methods

2.1. Sample Collection. Wastewater samples were collected on April 10, April 20, May 1, May 10, and May 20, 2022, from the wastewater tanks of three hospitals (Groups A, B, and C) and from the Huajiachi Area of Zhejiang University (Group S). A total of 20 samples were collected. The samples were stored in high-temperature sterilized 500 ml glass bottles. The samples were passed through a $0.2\,\mu m$ filter membrane using a vacuum negative pressure air pump. The filtered material was collected, placed in sterile microcentrifuge tubes, and stored at $-80°C$.
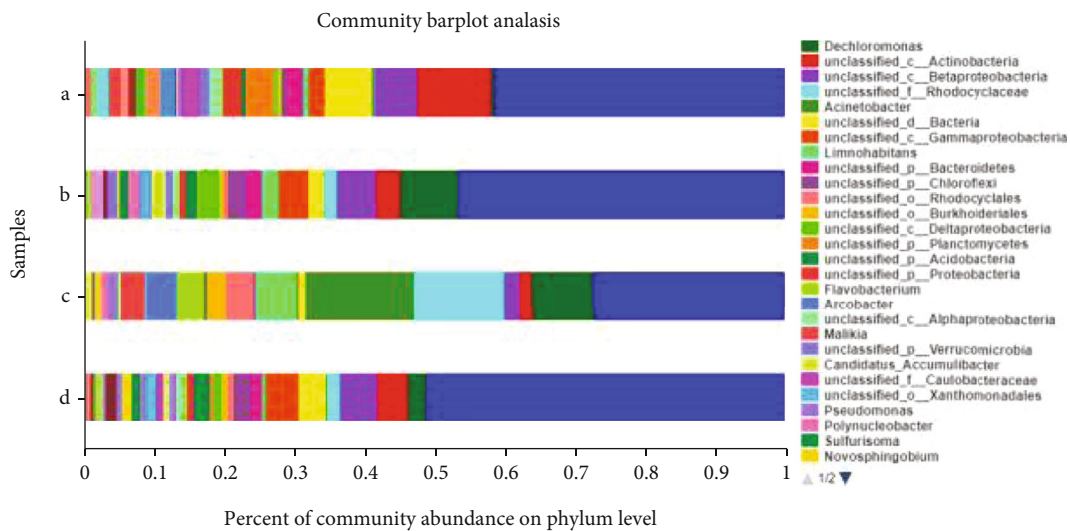
2.2. DNA Extraction, Library Construction, and Metagenomic Sequencing. Using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Buckhead, Georgia, U.S.A.), the total microbial genomic DNA was successfully extracted from the filter membrane material in accordance with the instructions provided by the manufacturer. A minifluorometer (TBS-380) and spectrophotometer (NanoDrop 2000) were used to examine the amount of extracted DNA as well as its degree of clarity. A 1% agarose gel (Analytik Jena) was used to determine the purity of the DNA extract. Using an ultrasonicator (Covaris M220, Gene Company Limited, China), the DNA strands in the extract were cut to an average length of 400 base pairs (bp) for them to be suitable for use in the establishment of paired-end libraries. A paired-end library was constructed using NEXTFLEX Rapid DNA-Seq (Bioo Scientific, Austin, TX, USA). The adapters were ligated to the blunt ends of the fragments after sequencing. All hybridization sites for the sequencing primers were included in the adapters. Paired-end sequencing was performed using an Illumina NovaSeq/HiSeq Xten (Genotyping Inc., San Antonio, California, U.S.A). Sequencing was performed at Majorbio Bio-Pharm Tech Co., Ltd., Shanghai, China (http://www.illumina

Community barplot analasis

(a)

Community barplot analasis

(b)

Community barplot analasis
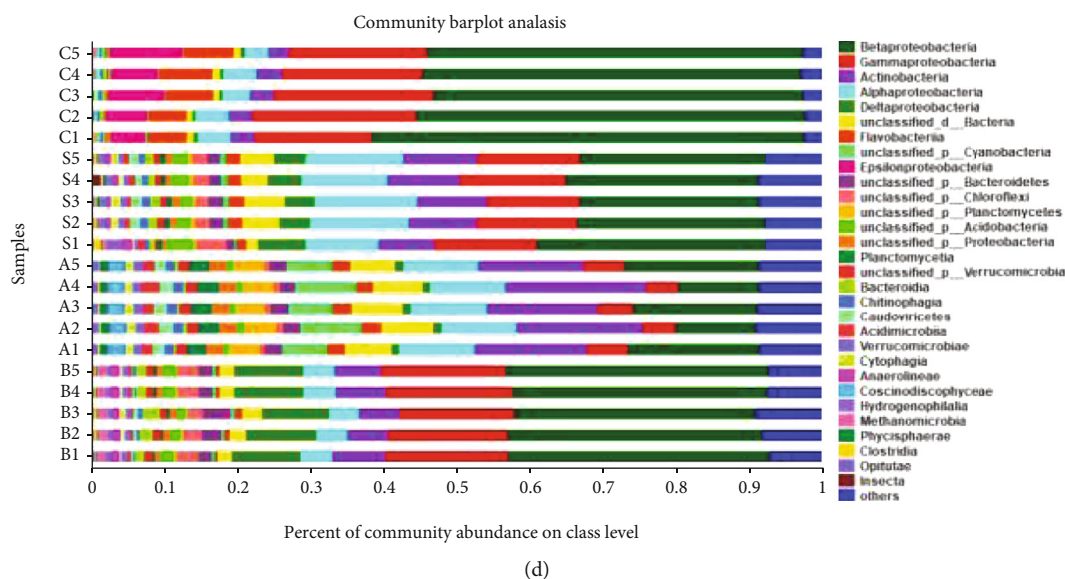

(c)

Figure 1: Continued.

Figure 1: Bacterial population composition in samples of wastewater. (a) Levels of phyla. (b) Levels of genera. (c) Levels of species. (d) Levels of classes.

.com). The sequencing information obtained in this study has been uploaded to the database of short reads housed in the National Center for Biotechnology Information (NCBI) database (ID: 11896123,PRJNA:866331).

2.3. Sequence Quality Control and Genome Assembly.

Data analysis was performed using the free online platform made available by Majorbio Bio-Pharm Tech Co., Ltd. in Shanghai (http://www.majorbio.com). The software Fastp (https://github.com/OpenGene/fastp, version 0.20.0) was used to filter out poor-quality reads from the paired-end Illumina sequencing data. Poor-quality reads were characterized as having a length of less than 50 bp, a quality value of 20. The analysis of the metagenomics data was performed using MEGAHI (version 1.1.2, https://github.com/voutcn/megahit). Condensed de Bruijn graphs were used. The assembly method resulted in the selection of contigs measuring 300 bp in length. Subsequently, the contigs were used for gene prediction and annotation.

2.4. Gene Prediction, Taxonomy, and Functional Annotations.

We generated hypotheses regarding the open reading frames (ORFs) of each generated contig using Meta-Gene (http://metagene.cbk.u-tokyo.ac.jp/). ORFs that were predicted to be less than 100 bp in length were collected, translated into amino acid sequences using a translation table (http://www.ncbi.nlm.nih.gov/), and subsequently evaluated. Using CD-HIT, a nonredundant gene catalog with 90% sequence identity and 90% coverage was created. We were able to estimate the relative abundance of genes by using SOAPaligner (version 2.21; http://soap.genomics.org.cn/), which enables the alignment of high-quality reads to nonredundant gene catalogs while maintaining 95% gene identity.

2.5. Species and Functional Annotations.

Diamond (version 0.8.35, http://www.diamondsearch.org/index.php) was used to align the sequences of the samples from a nonredundant gene catalog to the NR database in order to generate the taxonomy annotations. Diamond was run against the egg-NOG (*Evolutionary Genealogy of Genes: Non-supervised Orthologous Groups*) database (http://eggnog5.embl.de/#/app/home) to obtain COG (*Cluster of Orthologous Groups of proteins*) annotations for the representative sequences. KEGG (*Kyoto Encyclopedia of Genes and Genomes*) annotations were obtained using Diamond against the KEGG database (http://www.genome.jp/keeg/).

2.6. Antibiotic-Resistant Gene Annotations.

The CARD database (https://card.mcmaster.ca/home) is a free microbiome big-data database. Diamond (version 0.8.35, http://www.diamondsearch.org/index.php) was used to annotate genes related to antibiotic resistance, with an e-value threshold of $1e^{-5}$.

2.7. Data Analysis.

The Diamond tool was used to compare nonredundant gene sets with the NR library. The species identification results were obtained using the NR database. Subsequently, the abundance of the genes related to each species was calculated, and the complete abundance value for each species was used. The frequency of each species found in each sample was counted at the kingdom, phylum, class, order, family, genus, and species levels. To evaluate the nonredundant gene sets in relation to the Antibiotic Resistance Genes Database (ARDB), the Diamond program (https://github.com/bbuchfink/diamond) was used (parameters: blastp; e-value $1e-5$). The antibiotic resistance function that correlated with the gene ontology data was gathered, and the abundance of the antibiotic resistance function was derived based on the total number of genes found to be associated with that function. Sequences of the quel gene set were compared with those of the genes in KEGG using Diamond (https://github.com/bbuchfink/
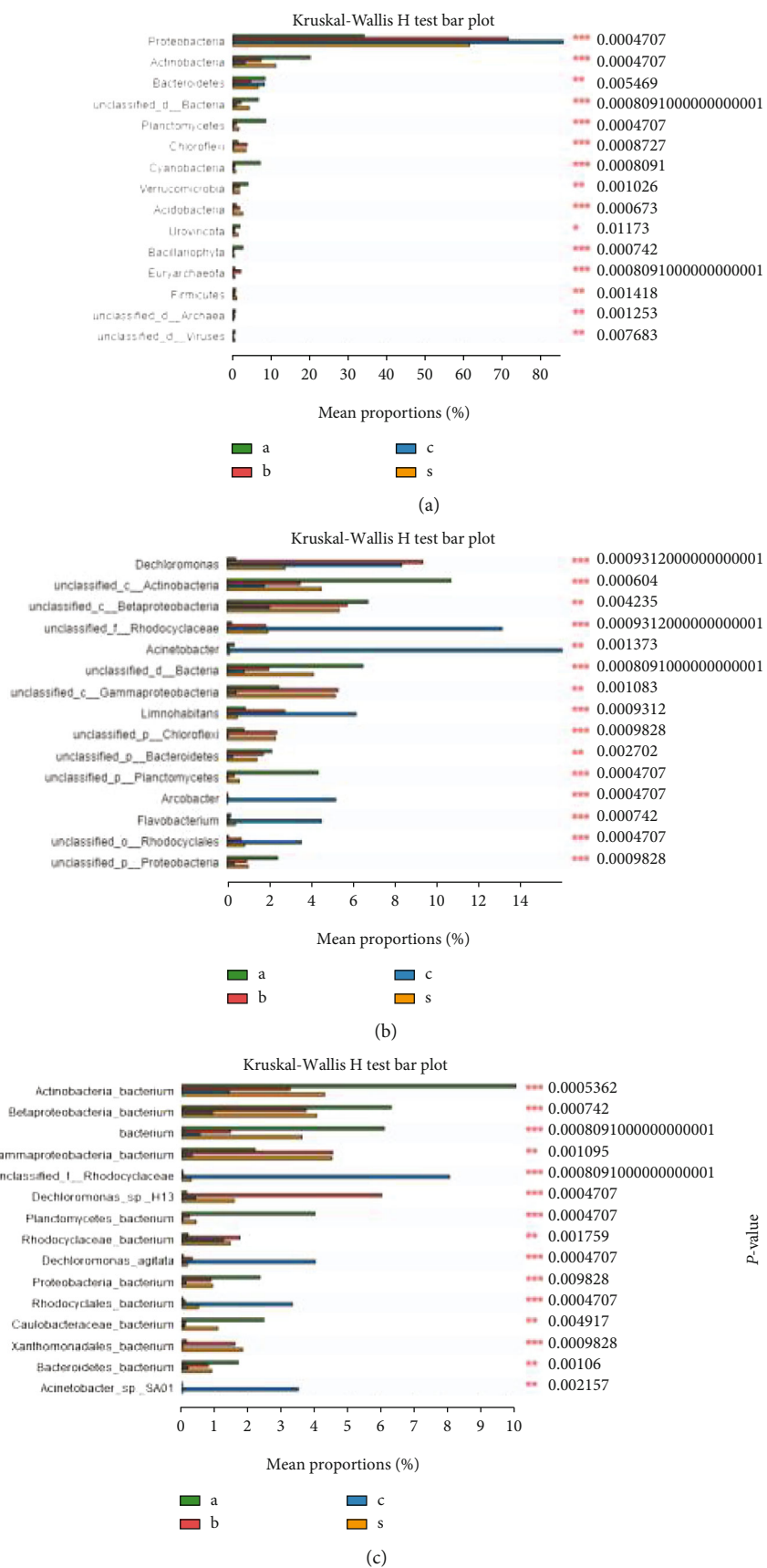
(a)
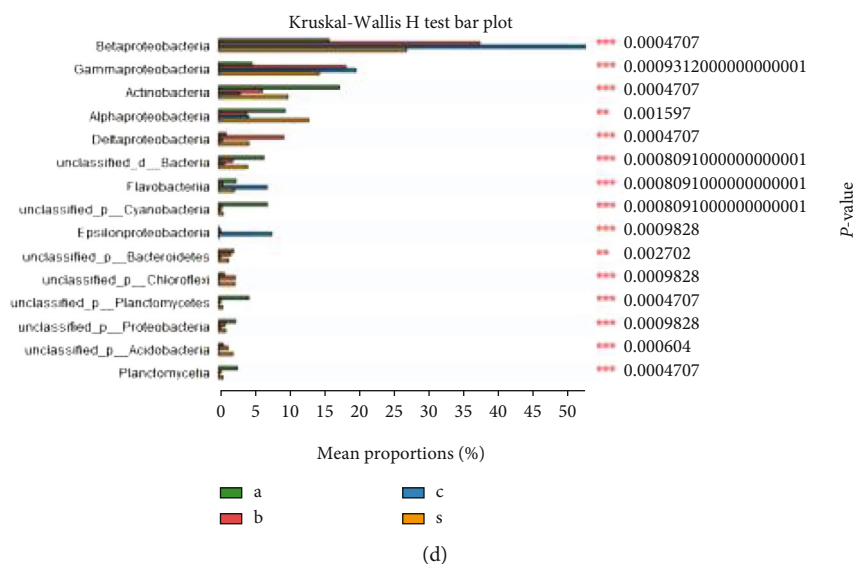

(b)

(c)

Figure 2: Continued.

Figure 2: Variations in the bacterial population composition at the (a) phylum, (b) genus, (c) species, and (d) class levels.

diamond). KEGG is an online database that contains microbe information on genes and genomes (parameters: blastp; e-value $1e - 5$) (parameters: blastp; e-value $1e - 5$). We estimated the abundance of class v by combining the abundances of the KO, pathway, EC, and module classes. The R scripting language was used to construct the figure. To illustrate the connection between the classes of antibiotics and microbial taxa groups, a cooccurrence network was implemented. Spearman's correlation was used to determine the significance ($P < 0.05$) and strength ($P > 0.5$) of the connections.

3. Results

3.1. Overview of Assembly and Annotations. A total of 1,762,675,816 clean readings were produced, with an average of 86,096,720 reads obtained for each sample. Statistical data for the sequences are shown in Table S1 in the Supplemental Materials. A BLASTP search was run against the eggNOG database to annotate the protein-coding genes detected in each DNA sample. The assembly of the metagenome, in addition to its annotations and the ORFs that were predicted for it, can be found in Supplemental Table S2. In order to generate the NR gene catalog, the predicted sequences of the genes detected in the samples were grouped together using CD-HIT. Additional information is provided in Table S3 in the Supplementary Materials.

3.2. Bacterial Community Characteristics in Wastewater Samples. The bacterial population in the wastewater was analyzed, and its composition was determined by comparing it to the relevant species and taxonomic annotation information stored in the cloud platform. The phylum composition between the four groups was completely different (Figure 1(a)). The samples were indicative of the bacterial populations in the wastewater; the analysis showed a total of 244 phyla and 6,415 taxa. A study of the bacterial community abundance in wastewater from different hospitals

revealed that Proteobacteria was the most abundant phylum in Group C (84.97%), whereas its abundance was relatively low in Groups A and B (34.53% and 67.82%, respectively). *Actinobacteria* was also the main phylum in Group A (18.16%), while the abundance of this phylum in Groups B and C was very low (7.23% and 3.26%, respectively). According to the data on genera, species, and classes (Figures 1(b)–1(d)), there were significant differences in the microbial community composition among the four groups. *Acinetobacter* (10.49%), *Dechloromonas* (8.43%), and *Acinetobacter* (15.49%) were the most abundant genera in groups A, B, and C, respectively. In contrast to hospital samples, all genera were uniformly distributed in domestic wastewater samples (Group S). Although the hospital in Group B is a TCM hospital, it is still a multispecialty hospital, like that in Group A, and receives all kinds of patients. This results in the presence of similar bacteria in the wastewater, but significantly different communities. However, it remains unclear whether this difference is caused by the administration of natural antibacterial herbs (traditional Chinese medicines). A stomatology hospital was included in Group C. The bacterial community of this specialized hospital was different from that of the two general hospitals, which has not been reported in previous studies. Group S included a university dormitory, and few drugs were administered in daily life, which caused an even distribution of microbial species (Figures 2(a)–2(d)). The samples contained numerous representatives of the phyla *Proteobacteria*, Actinobacteria, and *Bacteroidetes*. The average fractions of the 16 genera differed significantly ($P < 0.05$). There was no discernible difference between the aseptic genera. There were substantial variations in the microbial community composition across the four groups, according to the cluster analysis findings (Figure 3(a)). Groups A and B had more comparable microbial community compositions than Group C. To separate the samples from the center parallel axis 1 (PC1, 59.67%), the principal coordinates analysis (PCoA) was performed, and the same result was achieved as with
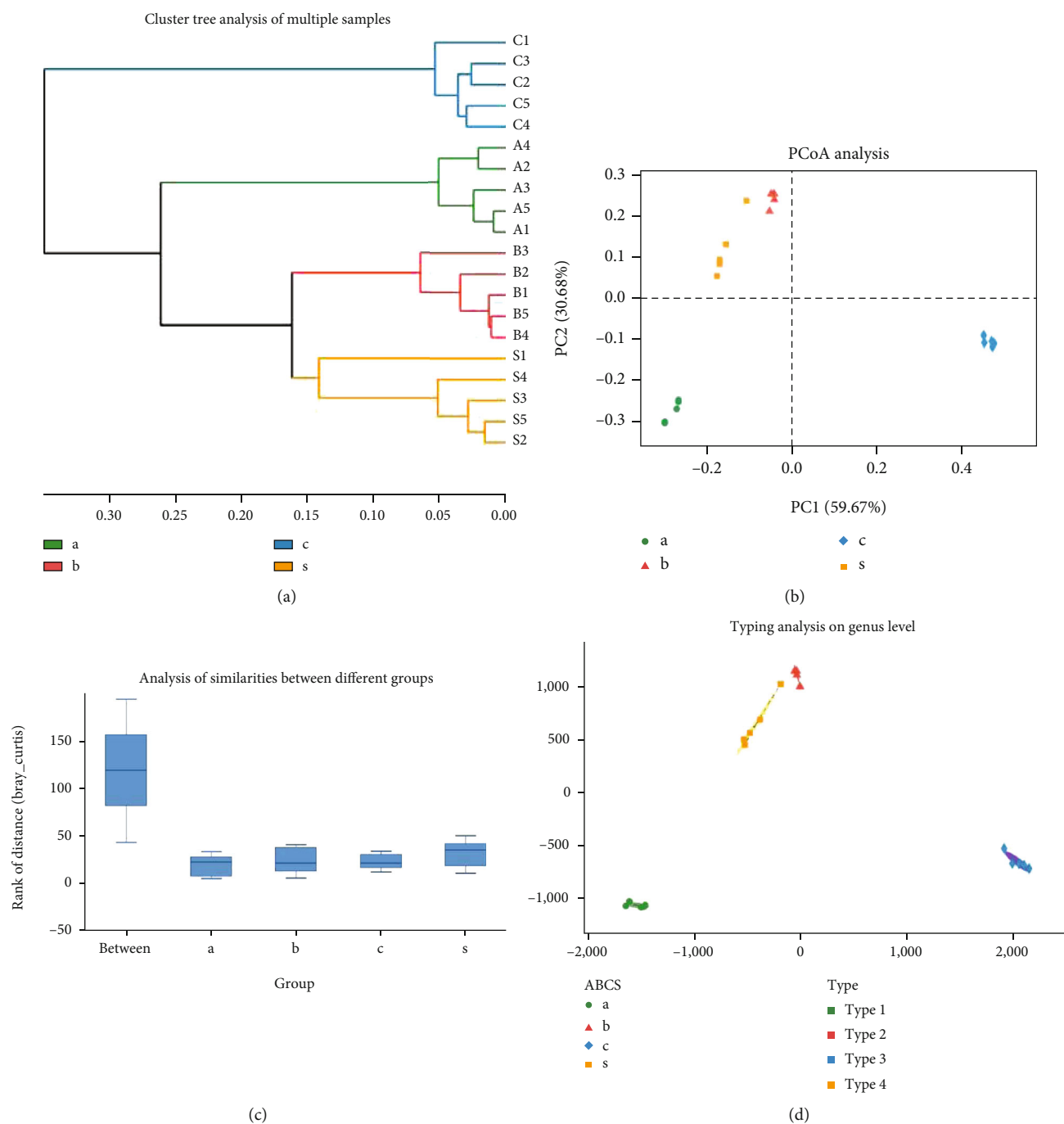
(a)

(b)

(c)

(d)

FIGURE 3: (a) Cluster analysis, (b) principal coordinates analysis, (c) analysis of similarities, and (d) typing analysis.

cluster analysis (Figure 3(b)). We also conducted a similarity analysis (Figure 3(c)), as well as a typing study (Figure 3(d)).The results are shown in the figure.

3.3. ARGs in Wastewater Analyzed by ARDB.
Using an ARDB comparison, 24 ARGs were identified among the four different groups. Figure 4(a) shows that the ARGs linked with bacitracin were the most abundant in wastewater from both Groups A and B. Besides bacitracin, sulfonamide and tetracycline were rated first and second in terms of abundance in the Group C effluent. In the PCoA, the abundance

of ARGs among the four groups was considerably different (Figures 4(b)–4(d)). Ten of the 15 primary ARG composition classes showed significant variation in all samples (Figures 5(a) and 5(b), $P < 0.05$). Furthermore, bacitracin, sulfonamide, and tetracycline were found to be the most abundant ARGs. Bacitracin was found in the greatest quantity in Group A ($P = 0.0004707$), whereas tetracycline was the most abundant in Group C ($P = 0.00969$). Sulfonamide abundance was the lowest in group A and was significantly different from the other groups ($P = 0.001095$). Furthermore, when comparing Group C to the other groups, there
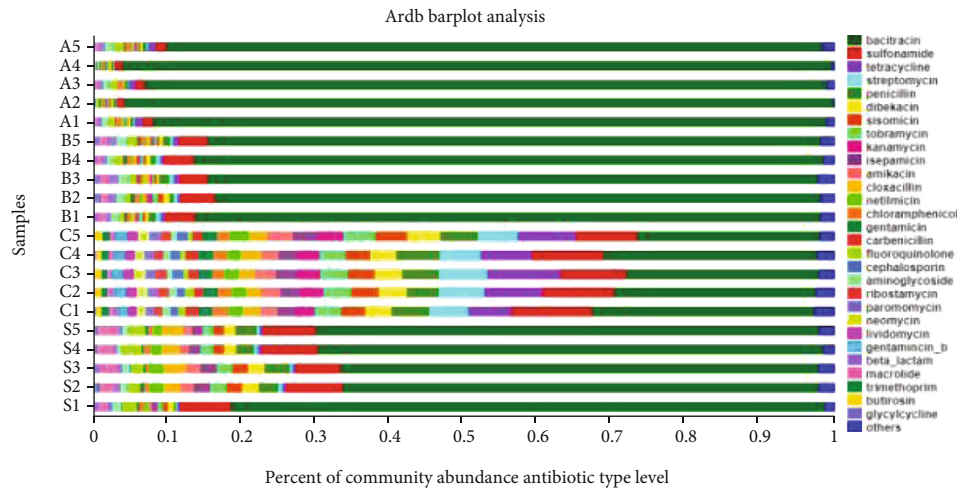
(a)
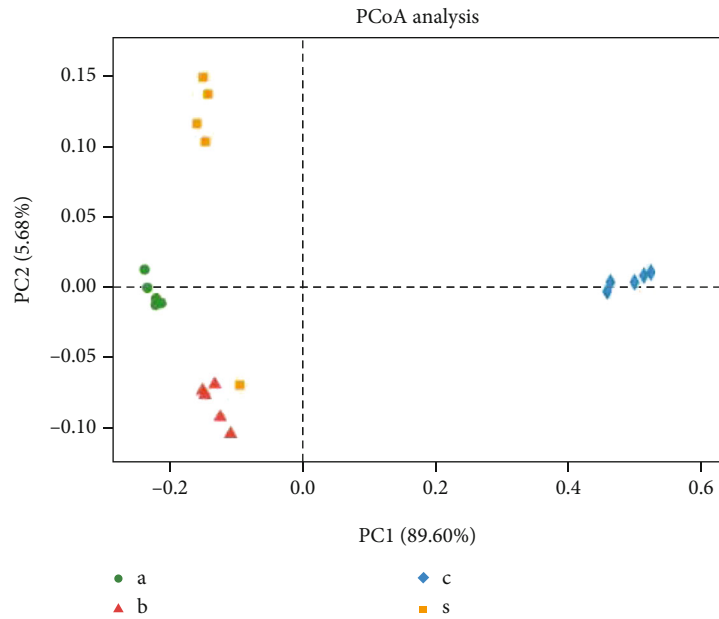
(b)
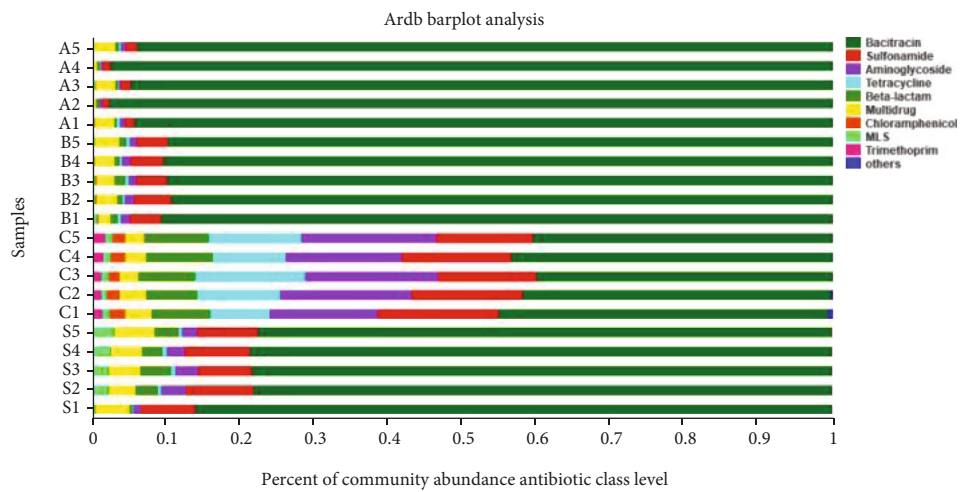
(c)

Figure 4: Continued.

Figure 4: (a) Percentage of the community's overall abundance based on antibiotic type ARG types. (b) PCoA of the ARGs. (c) Percent of community abundance on antibiotic class level. (d) Percent of community abundance on ARG antibiotic resistance gene level.

were substantial variations in the quantities of streptomycin, sisomicin, dibekacin, and tobramycin.
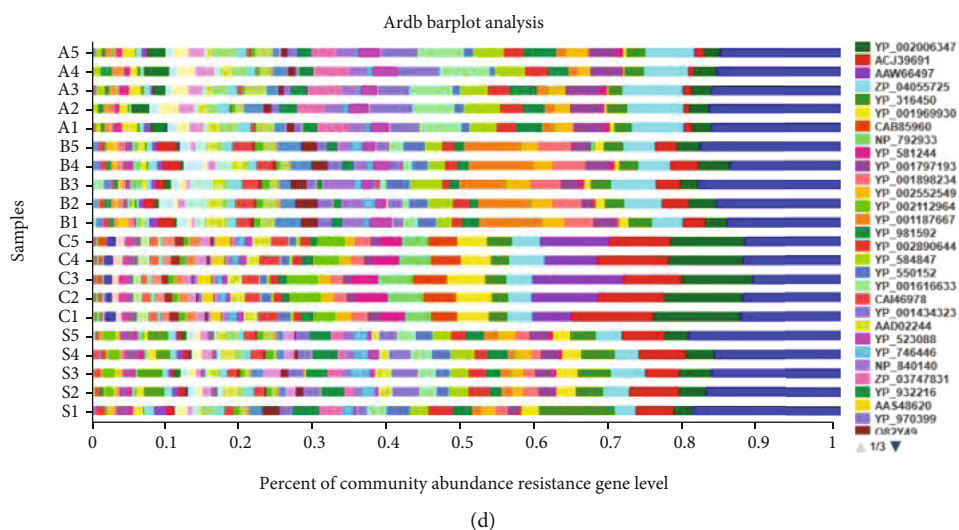
3.4. Total Bacterial Genes Analyzed by CARD. According to the CARD analysis findings, there were substantial variations in the types of ARGs in all the wastewater samples (Figure 6(a)). Genes related to antibiotic efflux accounted for the highest percentage of all samples. Between these four groups, there were additional differences in genes related to antibiotic target modification, protection, replacement, and inactivation (PC1, 69.62%, Figure 6(b)).The H test was used to examine the gene types in each bacterial community. Genes related to antibiotic efflux, antibiotic target change, protection, replacement, and inactivation accounted for the greatest percentage in all wastewater samples. For functional comparison of multiple groups of genes, differences in comprehensive ARGs are evaluated (Figure 6(c)). The above conclusions were validated by the heatmap analysis. Genes related to antibiotic efflux accounted for the highest proportion in all the samples. This indicates that all antibiotic-resistant microbes could activate antibiotic-resistant genes in all wastewater environments. Antibiotic inactivation/ target replacement was relatively weak in all groups. In addition, the α-diversity and β-diversity of species and function were calculated based on the species and functional abundance information of these samples. Linear regression analysis was also performed to assess consistency between species and function (Figure 6(a)). The abscissa represents the α- or β-diversity index of the function, and the ordinate represents the α- or β-diversity index of the species. A higher coefficient of determination (R^2) indicates a higher consistency in α- or β-diversity between species and functions. A negative coefficient of determination (R^2) indicates that there is no significance in the α- or β-diversity between species and functions. From the above figure, it can be concluded that there was poor consistency in Group C.

3.5. Species and ARG Abundance Correlation Analysis. Finally, network analysis was performed to explore the relationship between ARGs and microbes based on antibiotic type (Figure 7). A total of 10 ARGs cooccurred with 9 bacterial genera. Phodocyclaceae were positively correlated with sulfonamide, tobramycin, dibekacin, sisomicin, penicillin, isepamicin, tetracycline, and other antibiotic genes. Dechloromonas was positively correlated with tetracycline, isepamicin, penicillin, streptomycin, sisomicin, amikacin, and other antibiotic genes. Betaproteobacteria were negatively correlated with six ARGs (sulfonamide, dibekacin, sisomicin, amikacin, bacitracin, and penicillin). Gammaproteobacteria were negatively correlated with bacitracin, tetracycline, and isepamicin.

4. Discussion

There is a high concentration of antibiotics and presence of different pathogenic bacteria in the hospital wastewater, which may lead to the horizontal transfer of ARGs [16, 17]. Hangzhou, the provincial capital of Zhejiang Province, is densely covered by water reticulation systems. Therefore, the potential influence of wastewater from different hospitals on the environment should be studied carefully. In this study, a metagenomic method was used to analyze the differences in the bacterial composition of untreated wastewater from different hospitals in Hangzhou. Wastewater from a dormitory at Zhejiang University was selected as the control group (Group S). The three hospitals included in the study were a general hospital (FAHZU, Group A), TCM hospital (HZTCM, Group B), and stomatology hospital (Hangzhou Dental Hospital, Group C). Based on the NR database [18], the bacterial composition of wastewater from the three hospitals at the phylum and genus levels was significantly different than that of domestic wastewater. The bacterial composition of wastewater from the two general hospitals was similar at the phylum level and mainly included
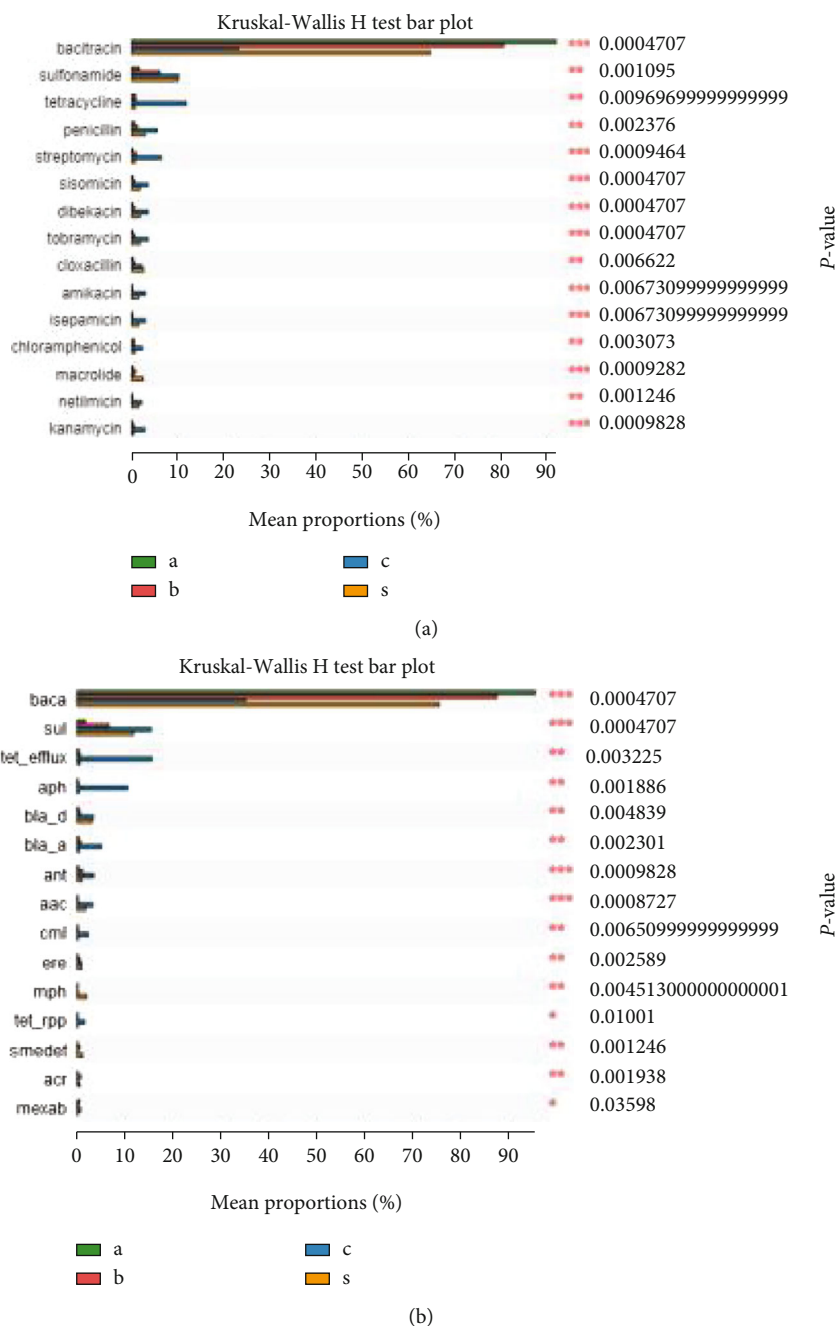
Kruskal-Wallis H test bar plot

bacitracin	*** 0.0004707
sulfonamide	** 0.001095
tetracycline	** 0.00969699999999999
penicillin	** 0.002376
streptomycin	*** 0.0009464
sisomicin	*** 0.0004707
dibekacin	*** 0.0004707
tobramycin	*** 0.0004707
cloxacillin	** 0.006622
amikacin	*** 0.006730999999999
isepamicin	*** 0.006730999999999
chloramphenicol	** 0.003073
macrolide	*** 0.0009282
netilmicin	** 0.001246
kanamycin	*** 0.0009828

P-value

Mean proportions (%)

a   c
b   s

(a)

Kruskal-Wallis H test bar plot

baca	*** 0.0004707
sul	*** 0.0004707
tet_efflux	** 0.003225
aph	** 0.001886
bla_d	** 0.004839
bla_a	** 0.002301
ant	*** 0.0009828
aac	*** 0.0008727
cml	** 0.00650999999999999
ere	** 0.002589
mph	** 0.004513000000000001
tet_rpp	* 0.01001
smedef	** 0.001246
acr	** 0.001938
mexab	* 0.03598

P-value

Mean proportions (%)

a   c
b   s

(b)

FIGURE 5: (a, b) Kruskal-Wallis *H* test bar plot components of ARGs.

Proteobacteria, Actinobacteria, and Bacteroidetes. However, the relative abundances in each group were different. The results of the clustering feature (CF) tree and PCoA of β-diversity of the bacterial community indicated that different sampling dates had little influence on the microbial structure of the samples. The β-diversity of the samples at one site was completely consistent [19], which may be attributed to the fixed sampling sites and small environmental variables.

The microbial community in the wastewater from the stomatology hospital was significantly different from that in the wastewater of the two general hospitals. The bacterial community composition in the wastewater of Groups A (general hospital) and B (general hospital) was similar to each other and was different than that of Group C. This may be explained by the fact that hospitals in Groups A and B are general hospitals equipped with relatively complete departments (including medicine, surgery, gynecology, pediatrics, and other routine departments) and have similar diagnosis and treatment activities. The hospital in Group C is a stomatology hospital specializing in stomatological practice and has different patient types, patient populations, and drug usage. This suggests that the hospital-acquired infection control departments of different hospitals should take corresponding wastewater treatment measures according to the bacterial composition of the wastewater from different hospitals [20]. Network analysis revealed that abundance
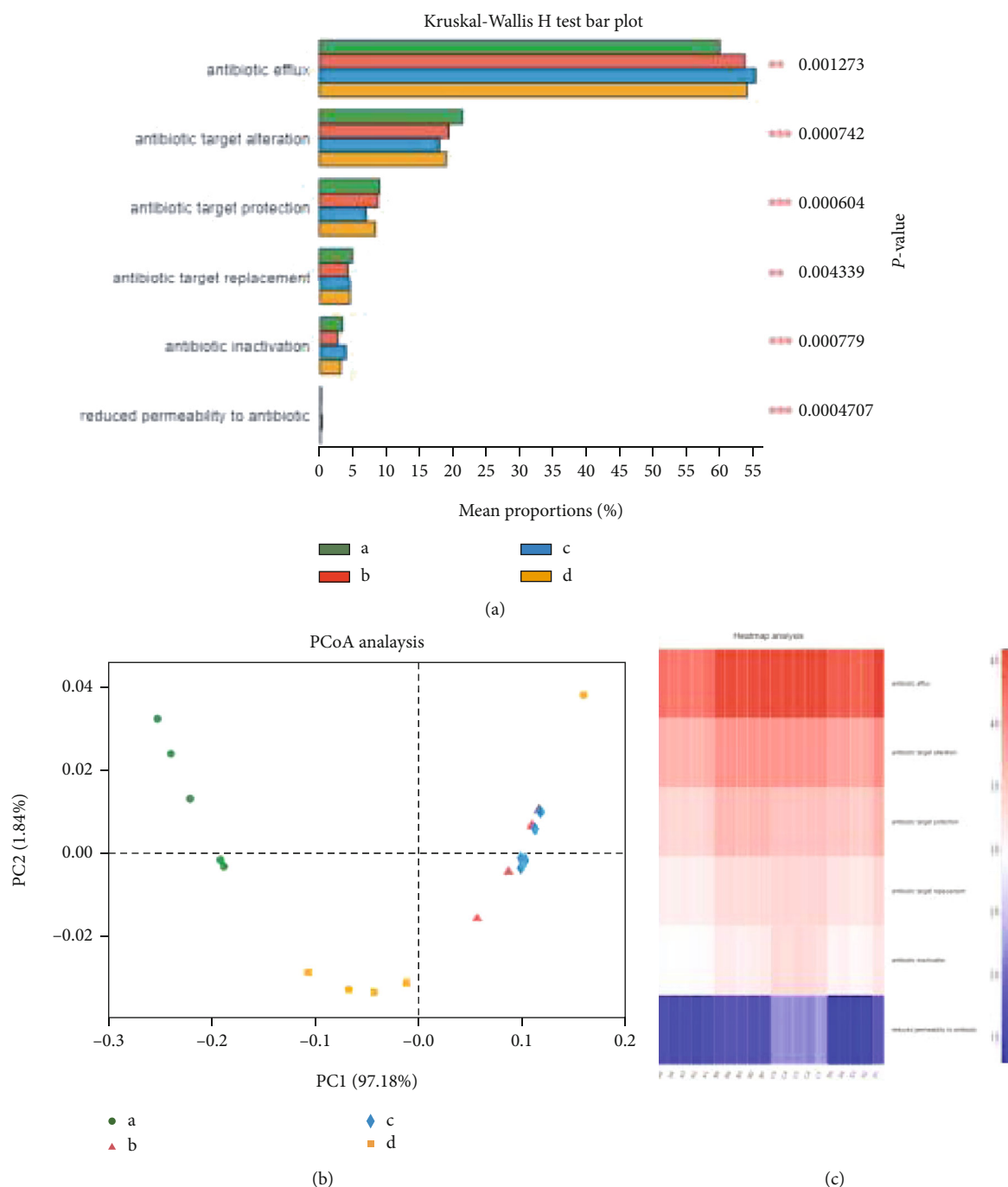
(a)

(b)

(c)

FIGURE 6: ARG types present in each sample of wastewater. (a) ARGs types. (b) PCoA of the gene types found in the bacterial community. (c) Functional comparisons between various groups of genes and numerous groups of genes.

of Phodocyclaceae was positively correlated with the presence of genes for resistance to sulfonamide, tobramycin, dibekacin, sisomicin, penicillin, isepamicin, tetracycline, and other antibiotics. Further, *Dechloromonas* abundance was positively correlated with presence of genes for resistance to tetracycline, isepamicin, penicillin, streptomycin, sisomicin, amikacin, and other antibiotics. Therefore, abundance of some species highly correlated with the presence of ARGs, such as Phodocyclaceae and Dechloromonas, should be specifically treated during wastewater

treatment. For instance, wastewater disinfection procedures should be specifically designed for eliminating bacteria from the genera Phodocyclaceae and Dechloromonas [21]. Moreover, Proteobacteria and Actinobacteria were highly abundant in hospital wastewater. Special attention should be paid to the significant changes in the abundance of such microbes in the environment during monitoring of urban planning departments, which could prevent environmental pollution caused by the leakage of untreated medical wastewater [22].
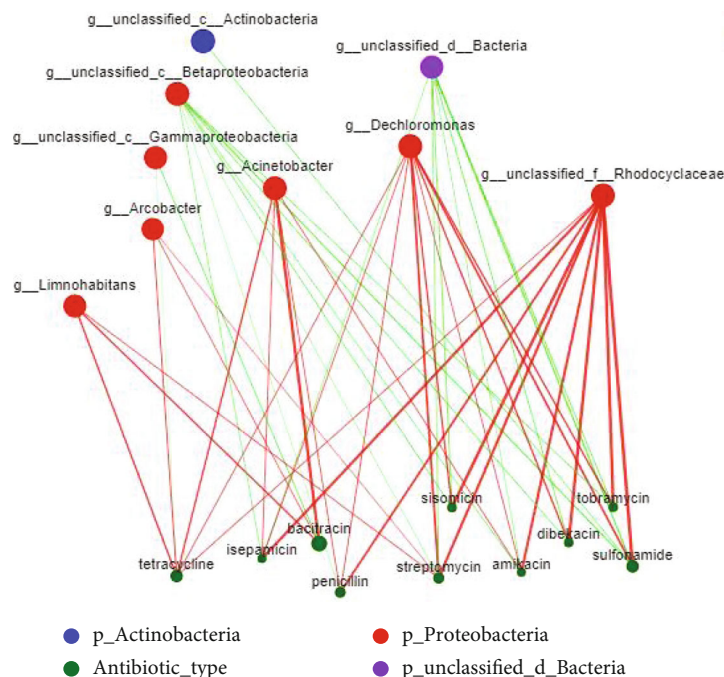
FIGURE 7: Correlation between microbes and ARGs based on antibiotic types.

Currently, wastewater mostly undergoes sewage treatment before it is discharged into the urban sewage system; this treatment can remove some antibiotic-resistant bacteria [23]. However, treated wastewater still contains a higher level of ARGs than that in natural water, and hospital wastewater is one of the main sources of ARGs in the natural environment. It is generally considered that clinicians in stomatology hospitals perform more manual treatments compared to those in general hospitals [24]. Antibiotic dosages prescribed in stomatology hospitals are not comparable to those in general hospitals [25]. Therefore, and the abundance and types of ARGs in wastewater from stomatology hospitals are expected to be low. However, our results contradict this assumption. The wastewater from the stomatology hospital contained ARGs from more categories than those from the other groups. However, bacitracin resistance genes were more abundant in wastewater from other hospitals than in the wastewater from the stomatology hospital. Among the 10 ARGs that were most abundant, six were significantly different between the three hospitals and one living area. Among them, resistance genes for the three most common antibiotics (bacitracin, sulfonamide, and tetracycline) were significantly different between the stomatology hospital and general and TCM hospitals. This may be explained by the fact that many antibiotic mouthwashes of different brands and doses are adopted in stomatology hospitals, which makes it easy for more antibiotics to enter wastewater directly. In contrast, antibiotics used in general and TCM hospitals may not come into direct contact with the wastewater. Therefore, specific disinfection and preventive measures should be adopted for treating wastewater from stomatology hospitals. Moreover, protocols should be established for disposal of mouthwash. Total ARG analysis was performed based on CARD. The results showed that antibiotic efflux was the most common gene category in the hospital wastewater. Furthermore, antibiotic target alteration, antibiotic target protection, and antibiotic target replacement were also common in hospital wastewater, and their composition was different among the four groups. This finding is in line with the results of the analysis of the bacterial population and ARG type.

The results of this study suggest that before hospital wastewater is discharged into the municipal sewage system, additional specific treatment techniques should be implemented to eliminate pathogens that may be present in wastewater [26]. However, the wastewater samples were collected during the same season, which does not adequately represent the bacterial composition throughout the year. Therefore, further samples collected over different times of the year could provide additional information regarding abundance of ARGs in hospital wastewater. In addition, there are no statistics available on the types of antibiotics that are present in raw wastewater from hospitals, despite the fact that there is an inverse correlation between the concentration of antibiotics and ARGs in wastewater. Thus, further studies on hospital wastewater must include research on related topics [27, 28]. In addition, strategies for preventing the spread of infection and pathogens through wastewater must be developed, and they must be based on the medical services provided by hospitals.

Data Availability

The data used in this study are available from the authors upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Materials

Table S1. Statistical data for the sequences.Table S2. Assembly of the metagenome, annotations, and projected open reading frames.Table S3. Sequences of genes that are not duplicated in the gene catalog (nonredundant). (*Supplementary Materials*)

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