Distribution Characteristics of Antibiotic Resistance Bacteria and Related Genes in Urban Recreational Lakes Replenished by Different Supplementary Water Source

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Abstract

The objective of this study was to investigate the distribution characteristics of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in urban recreational water with different water supply sources, 2 lakes as XQ and FQ which represented as surface water and reclaimed water were selected for the study. Water samples were collected from April to November in 2018 to investigate aspects including 8 physical and chemical indexes of waters, resistance degree of 5 different kinds of antibiotics, antimicrobial resistance heterotrophic bacteria content, resistance phenotype, antibiotic resistance gene types and antibiotic resistance strains of species distribution. The results showed that the changes of antibiotic resistance rate of heterotrophic bacteria in 2 lakes to 5 kinds of antibiotics were synchronous with time, and it would reach its maximum in autumn. The detection of 10 ARGs and *int1* gene in 80 strains screened out from 125 strains of heterotrophic bacteria showed that the detection rate of *tetG* and *tetA* genes was relatively high, especially *int1*, with the rate as high as 90%. In the detection of resistance spectrum, 51.25% of the resistance bacteria were double-resistance to AMP-CTX. The 80 isolate strains were of 9 genera and 19 species, among which *Bacillus cereus*, *Escherichia coli*, *Aeromonas veronii*, *Aeromonas caviae* and *Raoultella ornithinolytica* were the common ARB species in FQ and XQ. There was no relatively large differences in the content of ARB, detection rate, and identification of ARGs in 2 lakes. Correlation analysis was used to evaluate the association between the change of water quality indexes and the content of antibiotic resistance bacteria in 2 lakes, and it was found that the water temperature was significantly correlated with the content of ARB in Sulfamethoxazole and Cefotaxime ($p < 0.05$), while there were no other correlations between the changes of other water quality indexes and the content of ARB ($p > 0.05$).

1. Introduction

In recent years, antibiotic resistant bacteria (ARB) and Antibiotic resistance genes (ARGs) have got more attention, both as emerging water pollutant (Pruden et al., 2006). Indeed, both of them have been detected in lakes (Low et al., 2016), aquaculture farms (Gao et al., 2012), soil (Tan et al., 2019), wastewater treatment plant (McConnell et al., 2019), as well as in rivers and their sediments (Guo et al., 2018), surface water biofilm (Cynthia and Katharina, 2016) and sea sediments (Chen et al., 2013). Compared with the widespread existence of antibiotic resistant bacteria, antibiotic resistance genes is the fundamental reason for the wide spread of bacterial resistance. The spread of ARG in various environmental media through horizontal transfer greatly increases the situation of bacterial antibiotic resistance, posing a threat to the environment and human health. Water is closely related to human life and is an important part of people's production and life. According to some literature reports, however, water is likely to become the source and storage place of pathogenic bacteria and antibiotic resistant bacteria that are harmful to human health (Dong et al., 2019), and intensify their transmission (Fewtrell et al., 2015; Pang, et al., 2016). Previous literature reports mainly focused on fecal indicators, enterovirus, and several pathogenic bacteria such as salmonella in rivers, lakes, and others (Hlavsa et al., 2015). Compare with rivers and lakes, in urban areas with high population density, recreational water

such as fountains and landscape lakes provide excellent recreational facilities for urban residents (Helena et al., 2015). At the same time, urban recreational water is closely related to people's living. People will contact landscape water in the ways of boating. Also, urban recreational water plays an important role in the city water cycle, and most are connected to the urban river, in many cases, reclaimed water is used as a supply source. However, if there are pathogenic bacteria or other dangerous sources of antibiotic resistance genes in water, it will directly threaten public health. Therefore, it is extremely necessary to analyze the distribution of antibiotic resistant microorganisms in urban landscape water.

As urban recreational water has played a significant role in the continuous expansion of urbanization. At the same time, sufficient attention should be paid to the microbial pollution in the water. Although the urban landscape water develops rapidly at present, the source of water supply is an important problem in practice due to the constraint of its own characteristics. However, some studies have found that although the water source and sediment polluted by human beings have been further treated, it is still likely to play an important role in the re-pollution of water for recreational purposes (Elmahdy et al., 2016). Landscape water is replenished mainly by the surface waters. Due to the shortage of water resources, recycled water gradually as the first selection of urban landscape water supplements (Zhao et al., 2011) for its excellent cyclicity. But the current sewage treatment standards and technology are not able to make the sewage treatment plant to regenerate the processing of the ARB and ARGs effectively in the water. This could lead to reclaimed water has many ARB and ARGs, which might exacerbate the landscape water in the content of resistant bacteria and resistance genes. And then increase the risk of public safety of urban residents (Dong et al., 2019). With the urbanization process and the improvement of people's living standards, the existing role of urban landscape water will become much more important (Cui et al., 2019), and the application of recycled water in landscape water is becoming more and more common. The microbial pollution of recycled water also requires further study, especially whether the supply water sources of different will lead to increased risk of antibiotic resistant bacteria such as microbial, and pose a threat to human health. Research on the different supply water sources of urban landscape water in the ARGs and ARB is of realistic significance.

In this study, 5 kinds of common ARB patterns like: Tetracycline, Sulfa, Ampicillin, Ciprofloxacin, and β -lactams antibiotic resistance bacteria and some of its corresponding resistance genes as the research target. We took 2 different supply water sources of the landscape waters in Xi'an as the research object. compared their levels of resistant bacteria in water and the distribution of antibiotic resistance genes, in order to provide the reference for the control of microbial risk.

2. Material And Methods

2.1. Sampling site and sample collection

The landscape water selected in this study are XQ and FQ lake (Fig. S1, site 1: 108°59'24.04"N; 34°15'36.05"W; site 2: 108°54'24.44"N; 34°15'7.04"W) . They are located in the ancient city of Xi'an, which are the famous tourist attractions. A large number of tourists go to visit these 2 lakes every day.

Meanwhile, both lakes have a variety of water entertainment items, such as boating. People have frequent contact with landscape water. XQ lake has an area of about 100,000 square meters and an average water depth of 2 m, surface water is its source of water replenishment. The surface area of FQ lake is about 40000 square meters, with an average depth of 1.5 meters, using reclaimed water as its supply source.

The samples were collected from April to November 2018 in XQ and FQ lake, gather once a month, a total of eight months of the XQ and FQ lake respectively selected the sampling point 5 and 4 samples, they were respectively collected 40 and 32. 1 L water samples was collected below the surface of the water at 50 cm. All the Samples were mixed into a container. Then they were put in aseptic sampling bottles to take back in the laboratory within 6 h, preservation at 4°C. Experiments will be carried out within 12 h.

2.2. Water quality parameters

The contents of total nitrogen and total phosphorus in water samples were determined by the method described in the literature (Guo et al.,2018). After the samples were filtered through the filter membrane, the content of chlorophyll a in water samples was determined by the thermal ethanol method (Chen et al.,2006). The content of dissolved oxygen, pH, and water temperature were determined by reference to literature (Harnisz.,2013).

2.3. Content determination of heterotrophic bacteria and ARB

The heterotrophic bacteria and antibiotic resistant bacteria in water samples were counted by membrane method. The heterotrophic bacteria in water samples were counted by the filter membrane method (Novo et al.,2013). After gradient dilution of the water sample, the mixed cellulose filter membrane with a diameter of 0.45 microns was filtered by vacuum extraction. The bacteria trapped in the filter membrane were closely attached to the PCA culture plate and cultured at 30 °C for 24h. Colony count was conducted on the culture plate with a number of 20~200 colonies. The average number of colonies was used to calculate the number of heterotrophic bacteria per 100 mL of the water sample. According to the standards of the American clinical and laboratory standards association (CLSI2017) (Annette w. Fothergill.,2012), Ampicillin (AMP), Sulfamethoxazole (SMZ), Tetracycline (TET), Ciprofloxacin (CIP), and Cefotaxime (CTX) were added into PCA plates to prepare resistance plates, in which the mass concentrations of corresponding antibiotics were 32 µg/mL, 512 µg/mL and 16 µg/mL, respectively. Similarly, the content of 5 kinds of antibiotic resistant bacteria in the water sample was calculated by using the filter membrane method to determine the antibiotic resistant bacteria in the water sample.

2.4. Isolation and susceptibility testing of antibiotic resistant strains

Bacterial colonies were randomly selected from PCA plate and activated in LB broth. Then, the plates were crossed for isolation, purification, and preservation of bacterial strains. After using the K-B disc agar diffusion method, selection of sulfamethoxazole (SMZ 300 µg), tetracycline (TET 30 µg), ampicillin (AMP 10 µg), ciprofloxacin (CIP 5 µg), cefotaxime (CTX 30 µg), 5 kinds of typical antibiotics for heterotrophic

bacteria were isolated from the antibiotic susceptibility test, determination of inhibition rings diameter, with reference to the Clinical and Laboratory Standards Institute (CLSI) standards to determine the resistance pattern (Annette w. Fothergill,2015).

2.5. Detection of resistance, integron and strains sequencing analysis

According to the TaKaRa MiniBEST Bacteria Genomic DNA Extraction Kit Ver. 3.0 Kit instructions, the DNA Extraction and separation of antibiotic resistant strains and save, using 16S rDNA gene sequencing identification, universal primers using Bacteria 27F and 1492R (Harnisz et al., 2018), PCR technology was used to test. After amplification, the products were sent to Sangon Biotech(Shanghai) Co., Ltd. for DNA sequencing. The sequencing results were checked by Bioedit v7.0.9 software, and the obtained 16SrDNA sequences were compared in the GenBank database in the National Center for bio-technology Information (NCBI) to confirm the species.

According to literature reports, 10 kinds of antibiotic resistance genes were identified, including tetracycline resistance genes *tetA*, *tetB*, *tetG*, and *tetM*; Sulfanilamide resistance genes *su1*, *su2*, *su3*; β -lactam type of antibiotic resistance gene *bla*_{TEM}, *bla*_{CTX-M}, *bla*_{SHV}, and Class I integron gene *int1*. References of gene primer sequence (Zhang et al.,2014; Zhang et al., 2015; He et al.,2012: Luo et al.,2011) were synthesized by Sangon Biotech(Shanghai) Co., Ltd. Sequences of resistance genes and Reaction conditions shown in table 1.

The antibiotic resistance genes were detected by PCR with Reaction system: 25 μ L reaction system, template DNA 2 μ L, 10 $\times$ PCR Buffer 2.5 μ L, dNTP Mixture 2 μ L, TaKaRa Taq 0.2 μ L, upper/lower primers 1 μ L each, RNase-free Water 16.3 μ L. Reaction conditions: 95°C 8min, 95°C 30s, annealing 30s, 72°C 5min. The product was detected by 1% agarose gel electrophoresis (AGE).

Table 1 Primer sequences of the relative resistance genes and Reaction conditions.

Genes	Primer sequences(5'-3')	Annealing temperature (°C)	Length of fragment(bp)
<i>tetA</i>	F:GCTACATCCTGCTTGCCTTC R:CATAGATCGCCGTGAAGAGG	55	210
<i>tetB</i>	F:TACGTGAATTTATTGCTTCGG R:ATACAGCATCCAAAGCGCAC	61	206
<i>tetG</i>	F:GCACGCTGGTTTGGCTACA R:TGGCTGTGATTAGTCTCCTTGA	56	176
<i>tetM</i>	F:ACAGAAAGCTTATTATATAAC R:TGGCGTGTCTATGATGTTTAC	45	171
<i>su1</i>	F:CGCACCGGAAACATCGCTGCAC R:TGAAGTTCCGCCGCAAGGCTCG	56	163
<i>su2</i>	F:TCCGGTGGAGGCCGGTATCTGG R:CGGGAATGCCATCTGCCTTGAG	56	191
<i>su3</i>	F:CCCATACCCGGATCAAGAATAA R:CAGCGAATTGGTGCAGCTACTA	57	143
<i>bla_{TEM}</i>	F:AAGCTTATTAGCGTGT R:TGTCTATGATCTTGT	55	531
<i>bla_{CTX-M}</i>	F:GCTGGTTTCAGAA R:TGTGATTAGCTGT	55	869
<i>bla_{SHV}</i>	F:TGAATACGTTTTTAC R:TACCTTGTTATTCAC	55	295
<i>int1</i>	F:GGCTTCGTGATGCCTGCTT R:CATTCTGGCCGTGGTTCT	57	146
<i>16S rDNA</i>	27F:AGAGTTTGATCCTGCCTCAG 1492R:TACGGYTACCTTGTTACGACTT	56	1465

2.6. Data analysis

Using Microsoft Excel 2016 and SPSS 23.0 to process and analyze the data, and origin8.5 was used to process and optimize the data images. 2 independent sample tests (Mann-Whitney U test) were used to

analyze the difference between different samples, and Spearman correlation analysis was used to test the correlation between water quality index and antibiotic resistance rate of samples.

3. Results

3.1. Water quality indicators in 2 lakes

Eight water quality indicators of the 2 landscape waters were tested, including water temperature, dissolved oxygen, pH, chlorophyll a, total nitrogen, total phosphorus, nitrate nitrogen, and ammonia nitrogen, and the test results were shown in table 2.

Table 2 Water physical and chemical properties of each lake (mean values are in brackets).

Lake	Temperature (w) (°C) ^a	DO (mg/L)	pH	Chlorophyll - a (µg/L)	TN (mg/L)	TP (mg/L)	NO ₃ ⁻ - N (mg/L)	NH ₄ ⁺ - N (mg/L)
FQ	9.30-31.80 (21.59)	7.88- 11.37 (9.91)	8.14- 9.06 (8.66)	0.00-21.39 (7.08)	1.08- 5.47 (3.23)	0.02- 0.06 (0.03)	0.58- 1.24 (0.93)	0.02- 0.78 (0.29)
XQ	14.40-33.90 (24.43)	6.32- 9.58 (8.14)	7.67- 8.53 (8.19)	0.00-24.65 (9.42)	0.59- 2.93 (2.14)	0.04- 0.09 (0.07)	0.27- 2.19 (0.88)	0.00- 0.25 (0.12)

^aTemperature(w):water temperature

3.2. Distribution of antibiotic resistance rate

The temporal variation of antibiotic resistance rate 2 lakes are shown in figure 1. The variation trend of heterotrophic bacteria content in 2 lakes is similar, showing an increasing trend with time. The changing trend of antibiotic resistance rate of Tetracycline and Ampicillin in FQ lake was similar over time, and the number of Cefotaxime resistance bacteria had a larger change trend over time, while the change of antibiotic resistance rate of Sulfamethoxazole was less obvious, and the fluctuation of antibiotic resistance rate of ampicillin was more obvious. The antibiotic resistance rate of ampicillin and Cefotaxime in XQ lake showed the same trend with time, and the fluctuation was the most obvious, it reached its maximum antibiotic resistance rate value in July and September. The antibiotic resistance rate of Sulfamethoxazole was stable and reached the peak in September. By contrast, the antibiotic resistance rate of Ciprofloxacin tended to be stable as a whole, and the antibiotic resistance rate in autumn was higher than that in other seasons. In this study, the overall trend of the changes in the antibiotic resistance rate of bacteria in FQ and XQ water was similar, among which the content of ampicillin resistance bacteria in FQ lake was higher, and the antibiotic resistance rate fluctuated greatly with time.

The antibiotic resistance rate of ampicillin in FQ lake was the highest in September, which was 15.13%, the resistance rate of Cefotaxime and Ciprofloxacin reached the maximum value in July and August, which were 11.56% and 1.97% respectively. The detection rate of Sulfamethoxazole and Tetracycline resistance bacteria was lower. The antibiotic resistance rate of Sulfamethoxazole in XQ lake varied greatly, in summer it was higher than that in winter, reaching a maximum of 41.07% in September. Compared with FQ lake, the bacteria resistance to Cefotaxime and Ampicillin in XQ lake were relatively equal, with the highest antibiotic resistance rates of 13.96% and 12.32%, respectively. The overall trend of antibiotic resistance rate in 2 lakes was similar, and all showed that the antibiotic resistance rate in winter was lower than that in summer. Except for Cefotaxime resistance bacteria in FQ had the highest detection rate in July, the other 4 antibiotic resistant bacteria in 2 lakes all reached the maximum detection rate in autumn. It was found that the antibiotic resistance rates of Tetracycline and Ampicillin were lower in 2 lakes, while the resistance rates of the other 3 antibiotics were higher in 2 lakes. The antibiotic resistance rate in summer and autumn was higher than that in winter. Moreover, the antibiotic resistance rate of bacteria in 2 lakes was higher in August and September than in other months, presenting a significant upward trend.

3.3. Analysis of antibiotic resistance pattern and antibiotic susceptibility testing

Based on the detection of antibiotic resistance rate in 2 lakes, 5~8 strains were isolated and purified from each sample, and a total of 125 strains of heterotrophic bacteria were isolated, including 63 strains in FQ lake and 62 strains in XQ lake. Then 80 antibiotic resistance strains were screened out from heterotrophic bacteria according to judgment criteria (Annette w. Fothergill, 2015), including 41 strains in FQ lake and 39 strains in XQ lake. The resistance of 80 strains to 5 antibiotics was shown in table 3. As can be seen from the table, 80 antibiotic resistance strains had different degrees of resistance to 5 different antibiotics, among which the resistance to Ampicillin and Cefotaxime accounted for 93.75% (75/80) and 58.75% (47/80), respectively. However, the sensitivity to and Tetracycline was higher, with the proportion of antibiotic resistant bacteria being 26.25% (21/80) and 17.50% (14/80), respectively, with the lowest proportion of antibiotic resistant bacteria being 8.75% (7/80) to ciprofloxacin.

Table 3 antibiotic resistance phenotype of 80 strains were detected in 2 water

Antimicrobial agents	sensitive		antibiotic resistance	
	Number of strains	isolates%	Number of strains	isolates%
Ampicillin	5	6.25	75	93.75
Sulfamethoxazole	59	73.75	21	26.25
Tetracycline	66	82.50	14	17.50
Ciprofloxacin	73	91.25	7	8.75
Cefotaxime	33	41.25	47	58.75

The analysis of the antibiotic resistance pattern is shown in table 4. The experimental analysis shows that some antibiotic resistant bacteria show single antibiotic resistance, accounting for 13.60% (17/125) and 21.25% (17/80) of the isolated bacteria and the total number of antibiotic resistant bacteria. The antibiotic resistance phenotype of 70% single-antibiotic resistant bacteria is AMP, followed by SMZ. Most resistant bacteria showed resistance to 2 antibiotics, accounting for 40% (50/125) and 62.50% (50/80). The main antibiotic resistance phenotype of the second antibiotic resistance strain was AMP-CTX, and 41 strains were detected, accounting for 82% of all the double-resistance strains. 13 strains of multiple resistant bacteria, resistance to 3 kinds of common antibiotics, is more common AMP-SMZ-TET phenotype. Among which, 4 strains are resistant to 4 antibiotics, and 2 resistant to all 5 antibiotics. Multiple resistant bacteria resistant to 3, 4, 5 antibiotics were 8.75% (7/80), 5.00% (4/80), and 2.50% (2/80), accounting for the proportion of the total number of bacteria screened out were 5.60% (7/125), 3.20% (5/125), 1.60% (2/125). Among 80 strains, AMP-CTX is the most common resistance pattern, more than half of the isolates antibiotic resistant bacteria had this phenotype. On the whole, the number of strain antibiotic resistant pattern in FQ was higher than XQ, but the proportion of multiple resistant bacteria in XQ was higher than FQ, the only 2 strains which have 5 resistant patterns were from XQ, and it was found that the detection amount of multiple-antibiotic-resistant bacteria decreased with the increase of antibiotic types.

3.4. Occurrence of ARGs and integron in strains

The result of resistance genes detection of 80 ARB isolates from 2 lakes is shown in table 2. Tetracycline antibiotics resistance of bacteria 4 strains and 10 strains respectively. On the resistance gene detects found 2 lakes, *tetG* detection rate was 100% (4/4) and 50% respectively (5/10), known FQ lake *tetG* gene to their advantage. On the contrary, the detection rate of *tetA* in XQ lake was slightly higher than that in FQ lake (80% (8/10) and 75% (3/4), respectively. The detection rate of *tetB* and *tetM* was relatively low, both of the genes were 20% (2/10) in XQ , while neither of them was detected in FQ. In 2 lakes, the Tetracycline positive antibiotic resistant bacteria in FQ lake detected the corresponding resistance gene, and the detection rate of the positive strain resistance gene was 100%. 1 of the 10 strains of Tetracycline positive antibiotic resistance bacteria in XQ lake did not detect the corresponding antibiotic resistance gene.

Table 4 Antibiotic resistance patterns for 80 resistance strains in 2 lakes

Resistance to multiplicity	resistance phenotype	Source and quantity of samples (strains)		Total (plant)
		FQ	XQ	
# 1 ¹	AMP ²	6	6	12
	SMZ	3	2	5
	TET	ND	ND ³	0
	CIP	ND	ND	0
	CTX	ND	ND	0
# 2	AMP, SMZ	4	1	5
	AMP, TET	1	2	3
	AMP, CIP	ND	1	1
	AMP, CTX	24	17	41
# 3	AMP, SMZ, TET	2	3	5
	AMP, CIP, CTX	ND	2	2
# 4	AMP, SMZ, TET, CIP	ND	2	2
	AMP, SMZ, TET, CTX	1	1	2
# 5	AMP, SMZ, TET, CIP, CTX	ND	2	2

¹ #1: single resistance phenotype, #2:2 heavy resistance phenotype, # 3:3 heavy resistance phenotype, # 4:4 heavy resistance phenotype, # 5:5 heavy resistance phenotype.

² AMP: ampicillin, SMZ: sulfamethoxazole, TET: tetracycline, CIP: ciprofloxacin, CTX: cefotaxime.

³ ND: none detected

Among the 3 tested β -lactam resistance genes, FQ lake detected 2 types of resistance genes, *bla*_{SHV}, and *bla*_{TEM}, respectively, with the same detection rate of 12% (3/25). 3 of the 22 strains screened from XQ lake that are resistant to β -lactam antibiotics have detected the related antibiotic resistance gene. In addition to the above 2 genes, *bla*_{CTX-M} has also been detected. The overall detection rate of *bla*_{SHV}, *bla*_{TEM}, and *bla*_{CTX-M} in the 2 lakes is low.

In the detection of 3 Sulfa resistance genes of bacteria screened from FQ and XQ lake, it was found that *su*2 in FQ lake had the highest detection rate 26.83% (11/45), while *su*1 and *su*3 were the same 12.20% (5/41). The detection rate of *su*1 in XQ lake was 23.07% (9/39), and that of *su*2 was 15.38% (6/39), slightly higher than that of *su*3 12.82% (5/39). FQ and XQ lake respectively screened out 10 and 11

strains of sulfa resistant bacteria, and the corresponding detection rates of antibiotic resistance genes were 100% (10/10) and 90.90% (10/11), respectively. Compared with antibiotic resistance genes, the detection rate of *int1* in 2 lakes was the highest, over 90%.

3.5. Result of species identification

The identification of bacteria species is shown in figure 3a. It can be seen from the figure that the 80 strains of antibiotic resistant bacteria screened out from 2 lakes can be divided into 9 genera and 19 species, and there are certain differences among various antibiotic resistant bacteria. The dominant antibiotic resistant bacteria of Ampicillin and Cefotaxime are *Bacillus cereus*, while the antibiotic resistant bacteria of ciprofloxacin are few and only 6 types. The common species of Tetracycline resistant bacteria is *Escherichia coli*, *Bacillus cereus*, *Escherichia coli*, *Aeromonas veronii*, *Aeromonas caviae*, and *Raoultella ornithinolytica* are all antibiotic resistant bacteria in 2 lakes. Among the antibiotic resistant bacteria screened from the sample, *Bacillus cereus* occupies a certain number advantage over other bacteria, which showed that this kind of bacteria belongs to the main antibiotic resistant bacteria in landscape water, indicating that this bacteria may play a role in promoting the transmission, migration and transformation of antibiotic resistance genes. The identification of multiple resistant bacteria was shown in figure 3b. The results showed that *Escherichia coli* was the main strain of triple antibiotic resistant bacteria, accounting for 42.86%. *Raoultella ornithinolytica* was the main resistant strain. Among all multiple resistant bacteria, *Escherichia coli* has a higher detection rate, and it has detection in triple and quadruple antibiotic resistant bacteria.

3.6. Correlation between ARB and environmental variables

Correlation analysis was conducted between the detected concentration of antibiotic resistant bacteria and the synchronized water quality detection indexes of water temperature, dissolved oxygen, pH, chlorophyll a, total phosphorus, total nitrogen, nitrate nitrogen, and ammonia nitrogen. The results were shown in figure 4, where $p < 0.01$ indicated significant correlation and $p < 0.05$ indicated significant correlation. As can be seen from the figure, the water temperature was significantly correlated with the content of SMZ antibiotic resistant bacteria and CTX antibiotic resistant bacteria in 2 lakes, the total phosphorus content in FQ lake was significantly correlated with the content of ampicillin antibiotic resistant bacteria ($p < 0.05$), and the remaining water quality indexes were not significantly correlated with the concentration of antibiotic resistant bacteria.

4. Discussion

The major supplement source of urban landscape water is surface water, however, with the increasing shortage of water resources in recent years, many areas in China have gradually started to use reclaimed water from sewage treatment plants as the source of water replenishment for landscape water (He et al., 2016) to alleviate the increasingly exhausted water resources. However, at the same time, the removal of antibiotic resistant bacteria and antibiotic resistance genes in the regenerated water is not required for

treatment. If it is directly injected into the landscape water as supplementary water, it may cause pollution to the landscape water and thus affect human health.

4.1. Distribution characteristics of antibiotic resistant bacteria and resistant pattern

In the case of the change with time and season, bacteria in the FQ lake have different resistance to 5 kinds of antibiotics, 3 kinds of antibiotic resistance as a whole have the same change trend except for Ampicillin and Cefotaxime, whose resistance change with time and evident in the outside, and XQ had a similar situation. There is a special phenomenon in July to September, during this time, antibiotic resistance rate in 2 lakes in all percentages increased greatly, the maximum resistance to basic all appear at this time, especially concentrate in September. It may be related to the rainfall in Xi'an during that period. Rainfall is likely to help antibiotic resistant bacteria and antibiotic resistance genes to landscape water. Studies have found that rainfall will increase the abundance of antibiotic resistant bacteria and antibiotic resistance genes in a storm drain (Ahmed et al.,2018), thereby causing microbial pollution to downstream water (Newton et al.,2019). At the same time, reclaimed water is used to irrigate the soil around the landscape water, resulting in certain antibiotic resistance genes and antibiotic resistant bacteria in the soil (Wang et al.,2014; Han et al.,2016), under the scour of rain, these soil pollutants will enter the landscape water, thus increasing the content of antibiotic resistant bacteria in the water. On the detection of resistance rate, FQ and XQ lake also appear similar situation, SMZ, AMP and CTX antibiotic resistant bacteria detection rate is higher than that of TET and CIP, the AMP and CTX antibiotic resistant bacteria detection rate is on the top, Whole detection rate order from high to low was AMP > CTX > SMZ > TET > CIP. Experiments of water resistance phenotype from the 2 out of 80 strains also side confirmed the results. The existence of antibiotics in water will lead to resistance of bacteria in water under pressure selection (Xu et al.,2016). Meanwhile, the antibiotic resistance rates in 2 lakes were found to be high in summer and winter on the whole, which was similar to the situation in the other study (Huang et al.,2019). In this study, a content difference test of 5 types of antibiotic resistance bacteria was conducted and the results found that 3 kinds of antibiotic resistance bacteria content (Ampicillin, Ciprofloxacin and Sulfa) are not relatively large differences It can be concluded that 2 lakes of different water supplement water sources will not lead to antibiotic resistant bacteria content in the landscape water are different.

Based on the detection of antibiotic resistance rate in the early stage, 80 strains of antibiotic resistant bacteria were screened out from 2 lakes through an antibiotic sensitivity test, and the distribution of antibiotic resistant patterns of these antibiotic resistant bacteria was very similar. The phenotype resistant to AMP-CTX is the most, accounting for more than half of the antibiotic resistant phenotype, which is a very interesting phenomenon. In previous reports, resistance to 1 antibiotic is more common, while such a wide range of double-resistance is rare. Moreover, in this study, the AMP-resistant phenotype was common and detected in almost all antibiotic resistant bacteria, which indicated that AMP-resistant phenotype was dominant in 2 lakes.

4.2. Distribution characteristics of ARGs and class $\int$ integron in 80 antibiotic resistant strains

PCR was performed on 10 antibiotic resistance genes and class β integron. Tetracycline antibiotic resistance genes had a high detection rate, among which *tetA* and *tetG* genes were the main coding genes. Bacterial resistance to antibiotics mainly develops resistance to antibiotics through changing the target of antibiotic action, efflux pumps antibiotic mechanism, inactivating or inactivating enzymes of antibacterial antibiotics, changing pore protein to prevent antibiotic penetration, and changing antibiotic metabolic pathways (Tong et al., 2012). However, the occurrence of tetracycline antibiotic resistance is generally due to the efflux pumps shielding target or the enzyme inactivation required by antibiotic antibiotics. The mechanisms of the two Tetracycline resistance genes with the highest detection rate are both efflux pumps mechanism, and efflux pumps mechanism is the most common antibiotic resistance in tetracycline resistance. Studies on winter rivers in Shijiazhuang, China found that *tetA* and other genes were detected in natural water (Luo et al., 2019), and the detected amounts in rivers and sediments were different. Some studies have found that Sulfonamides, tetracycline and integron genes are ubiquitous in the sediments in the estuary and coastal areas of the Yangtze river (Lin et al., 2015), and the content of Sulfonamides, Tetracycline and integron genes show a trend of decline from the estuary to the coast. It can be found that Tetracycline resistance genes are quite widespread, and efflux pumps Tetracycline resistance genes are dominant. The detection rate of β -lactam resistance genes was relatively low, except for a small number of detected genes, most antibiotic resistance patterns strains did not detect the corresponding genes, which was consistent with some studies (Jiang et al., 2019). It is speculated that in addition to the tested genes, there may be other corresponding resistance genes (Knapp et al., 2012). However, in this study, only 3 types of β -lactam resistance genes were selected, which makes the solid detection has certain limitations. It was also found that although Tetracycline and Sulfonamides resistance genes were detected in both antibiotic resistance bacteria in 2 lakes, the prominent points of these 2 antibiotic resistance genes were different. The *tetG* had the highest detection rate of Tetracycline in the FQ lake, and Sulfonamides had the highest *su/2* rate, while XQ lake corresponds to *tetA* and *su/1*. This may be related to genetic original mobile *int* higher detection rate, *int* as the carrier, in the dissemination of resistance genes play an important role (Uyaguari et al., 2013). Researchers have found that the *int* in natural water such as the river has a high correlation with antibiotic resistance genes detection rate and a low correlation in the wastewater treatment plant (Ma et al., 2017). Although suspended substances in water were not detected in this study, suspended substances in landscape water, such as algae and micro plastics, might influence the spread of antibiotic resistance genes together with integron (Fang et al., 2018; Fang et al., 2018). The test of *int* detection rate more than 90%, it is highly possible that rainfall also led to a increase in the content of integrons in lake, leading to the difference in the major antibiotic resistance genes found in the 2 lakes, which is similar to some studies (Chen et al., 2019), and the detailed reasons need to be further studied.

4.3. Distribution characteristics of antibiotic resistance strains genera

The identification of 80 strains of antibiotic resistant bacteria showed that *Bacillus cereus* and *Escherichia coli* were the main strains. *Bacillus cereus* as a kind of conditionally pathogenic bacteria can cause food poisoning. It can be detected in the food and clinical scenario, but little document reported it in water environment (Zhuang et al., 2013). The reports on the distribution of *Bacillus cereus* in the water-

supply plant were studied and we found that conventional technology has a better removal effect (Jiang et al., 2018). Other studies tested the antibiotic resistance of 60 strains of food-borne *Bacillus cereus* in Guizhou, China, and found that they were resistant to Ampicillin, Trimethoprim, and Cefepime, which could not be used as clinical therapeutic antibiotics for food-borne diseases caused by *Bacillus cereus* (Zhou et al., 2019). Humans will be exposed to these Landscape water to a large extent, and may even inhale aerosols containing antibiotic resistance bacteria (Pal et al., 2016), which may increase the possibility of human infection with pathogenic bacteria. As an intestinal microorganism, *Escherichia coli* has been monitored as a water pollution indicator, and excessive drainage in sewage plants will lead to the increase of antibiotic resistant *Escherichia coli* in water (O 'flaherty et al., 2019). Recent studies have shown that the spread of antibiotic resistance genes in water may be mainly caused by faeces, rather than the environmental pressure selection (Karkman et al., 2019), so the most common type of *Escherichia coli* in excrement and urine, are thought to be most likely to spread as a source of bacteria. The *Escherichia coli* existing in the human intestinal tract, its resistance can be transferred to those bacteria of intestinal symbiotic medium which were originally sensitive to antibiotics (Ham et al., 2012). In this study we found in the detection of multiple resistance, *Escherichia coli* were resistant to AMP and other 3 kinds of antibiotics, but only sensitive to CTX, no CTX resistant *Escherichia coli* existed. This may signal that *Escherichia coli* has a high sensitivity to CTX. It may also be the reason that CTX resistance gene detection rate is low in 2 lakes leading to a low shifting level, detailed reason needs further exploration. Most of the remaining identified antibiotic resistant bacteria are not pathogenic bacteria, but they can transfer antibiotic resistance genes to pathogenic bacteria through horizontal gene transfer. Tests also found a small amount of other pathogenic bacteria such as *Pneumonia klebsiella*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, they exist in landscape water, providing opportunities that the pathogenic bacteria may be possible to acquire resistance genes. Conditional pathogenic bacteria and pathogenic bacteria, after enters the human body, can increase the use of antibiotics of treatment, cause multiple antibiotic resistance superbug to appear (Alicia et al., 2018), pose a potential health risk for humans. Bacterial resistance can be divided into intrinsic resistance and acquired resistant. In this study, among 80 antibiotic resistant bacteria, most of them were acquired resistance, few of them were intrinsic resistance. 9 strains were identified as intrinsic resistance, including 3 genera like *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia*, which needs to be further enriched by subsequent studies.

4.4. Effects of water quality indexes on the content of antibiotic resistant bacteria in landscape water

The physical and chemical properties of water as a routine indicator may affect the situation of antibiotic resistant bacteria in water. However, in this analysis of the correlation between water quality detection indexes and antibiotic resistant bacteria, except that the change of water temperature correlated with the concentration of antibiotic resistant bacteria in SMZ and CTX in 2 lakes, only the content of total phosphorus had a significant impact on the concentration of antibiotic resistant bacteria in ampicillin in lake FQ. The remaining water quality indexes do not affect the concentration of antibiotic resistant bacteria in the 2 lakes. The content of ampicillin antibiotic resistant bacteria in the water can be reduced by controlling the content of total phosphorus.

5. Conclusion

The results of this study showed that antibiotic resistant bacteria and antibiotic resistance genes were detected in 2 different landscape waters. Moreover, it was found that the surface water nor the reclaimed water was used as the replenishment source of the landscape water, it would not affect the species and content of antibiotic resistance genes and bacteria in the landscape water, indicating that the reclaimed water as the replenishment source was not the transmission source of antibiotic resistance genes. Through the identification of microbial species, we found that the antibiotic resistant bacteria in landscape water were highly concentrated in several types of bacteria. Moreover, it was found through analysis that the removal of total phosphorus was correlated with some antibiotic resistant bacteria, and the changes of other conventional water quality indexes would not affect the content of antibiotic resistant bacteria. The results of this study can provide references for further assessment of the risk of antibiotic resistant bacteria and antibiotic resistance genes in landscape waters and the impact of regenerated water on water replenishment.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YQL sorted out all the data and writing the manuscript; CMZ provided the idea of this study and was a major contributor in writing and modifying the manuscript; XM analyzed the water samples.; PPZ

collected the water samples.; JL help the investigation; ZW help the investigation. All authors read and approved the final manuscript.

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Figures

Figure 1

Temporal changes of 5 kinds of antibiotic resistance bacteria rate and total heterotrophic bacteria changes in water taken along the FQ and XQ lake (a):SMZ;(b):TET;(c):CIP;(d):AMP;(e):CTX;(f):Total heterotrophic bacteria.

Figure 2

ARGs and class 1 integron distribution of 80 antibiotic resistant bacteria isolated from FQ and XQ lake. The prevalence of (a) Tetracycline resistance genes; (b) Sulfa resistance genes; (c) β -lactam resistance genes; (d) class 1 integron

Figure 3

a. Identified genera containing 80 antibiotic resistance bacteria. b. Identified genera of multiple-antibiotic resistance bacteria. 3 pattern: 3 antibiotic resistance patterns; 4 pattern: 4 antibiotic resistance patterns; 5 pattern: 5 antibiotic resistance patterns.

Figure 4

Spearman correlations among antibiotic resistance bacteria abundance and water quality parameters in 2 lakes. (a):FQ lake;(b)XQ lake.

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