Spatiotemporal variation of bacterial communities in three cascade reservoirs in a southern city of China
Guangqiang Cai, Tongzhou Liu, Jinsong Zhang, Haoran Song, Xiaoyan Lu and Lijun Liu

ABSTRACT
Reservoirs are important water sources of urban drinking water supply. Bacteria play an important role in the removal of organics in reservoirs, but some of them can pose a health risk to consumers. Knowledge of bacterial community composition in drinking water sources can favour the drinking water security safeguards. This study investigated the monthly composition and diversity of bacterial communities in three cascade reservoirs using high-throughput Illumina HiSeq sequencing over one hydrological year. The diversity and structure of the bacterial community showed distinct spatiotemporal variation. Mycobacterium, Acinetobacter, Sphingomonas, Sphingobacterium and Pseudomonas were the genera resistant to chlorine. In addition, Acinetobacter, Arcobacter, Mycobacterium, Pseudomonas and Staphylococcus were the main potential pathogenic genera. The bacterial community diversity and the average relative abundance of potential pathogenic genera detected in the wet season were higher than those in the dry season. Water temperature was found to be the main factor for the temporal variation. The spatial variation of bacterial community diversity and the average relative abundance of potential pathogenic genera were related to water current and the degree of water pollution, respectively. The results of this study can conduce the beneficial management of drinking water treatment processes.

Key words | bacterial community, cascade reservoir, drinking water, Illumina HiSeq sequencing, potential pathogenic bacteria

HIGHLIGHTS
- Bacterial communities in three cascade reservoirs showed spatiotemporal variation.
- Bacterial diversity and potential pathogenic genera abundance were higher in the wet season.
- Water temperature was the major factor leading to the temporal variation.
- The spatial variation was related to water current and the degree of water pollution.

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INTRODUCTION

Reservoirs are important water sources of urban drinking water supply in China (Yang et al. 2015), especially for the southern city (subtropical area) in the present study, whose source water is almost completely from cascade reservoirs. Bacteria are an important component in aquatic ecosystems of reservoirs, and they play a vital role in the biochemical cycle of nutrients and biological transformation of organic matters (Newton et al. 2011; Crespo et al. 2013; Liu et al. 2017). There are also some potential pathogenic bacteria in reservoirs, for instance, some *Acinetobacter*, *Legionella* and *Mycobacterium* species (Yu et al. 2014). With the development of economy and society, water treatment processes have gradually improved and can deal with the risks in most cases. However, bacterial risks occur in some drinking water treatment plants in the southern city, which are mainly related to the change in bacterial community in the source water. Nevertheless, the bacterial communities in the cascade reservoirs remain unclear. A full understanding of composition, diversity, seasonal variation and influential factors of bacterial communities in the cascade reservoirs is therefore of great importance for the selection, operation and management of subsequent water treatment processes, which is vital for ensuring the bacterial safety of the drinking water supply.

There are a number of studies on the analyses of bacterial communities in many drinking water sources, including rivers (Kolmakova et al. 2014; Liu et al. 2018b; Adhikari et al. 2019), lakes (Bai et al. 2015; Ji et al. 2018, 2019), reservoirs (Wen et al. 2013; Wei et al. 2014; Yu et al. 2014) and groundwater (Chen et al. 2019). However, it is regrettable that most of those studies only involved one drinking water source and/or were conducted once, which did not reflect the spatiotemporal variation of the bacterial community. Only a few studies investigated the potential pathogenic bacteria, which not only pose a threat to human health, but may also cause pipe corrosion if they enter the distribution system. Moreover, even though the influence factors on composition and diversity of bacterial community have been studied in lakes (Niu et al. 2018; Ji et al. 2019) and rivers (Liu et al. 2015; Adhikari et al. 2019), those on the bacterial community and potential pathogenic bacteria in cascade reservoirs remain unknown.

In this study, a series of water samples were collected on a monthly basis from a group of three cascade reservoirs in a southern city in China over the course of a whole hydrological year. The bacterial communities, potential pathogenic bacteria and their relationships with water characteristics were investigated. Furthermore, the spatiotemporal variations in community diversity, potential pathogenic bacteria and the influencing factors were determined. The results of this study can contribute to the drinking water security safeguards, and can provide a reference for other cascade reservoirs used as drinking water sources in the subtropical area.

METHODS

Sample collection and physico-chemical analysis

The three cascade reservoirs (YT, SZ and SY) are the major drinking water sources for a southern city of China...
(subtropical area), and they provide a daily raw water supply of 3,600,000 m³ for drinking water treatment plants and serve almost seven million people. Their raw water originates from the Dongjiang River, which is a branch of the Pearl River. The raw water flows into three cascade reservoirs (YT–SZ–SY) through pumps and pipelines (Figure 1). Their basic information is provided in Table S1. In this study, 36 water samples were collected from the water intakes for the water plants of the three reservoirs from April 2017 to March 2018. During this time, the wet season was from April to September in 2017 and the dry season was from October 2017 to March 2018. All water samples were denoted as the name of sampling reservoir plus the month of sampling, and each reservoir’s samples were divided into a water sample group. Water samples were taken in 10-L sterile bottles, and each original sample was from the mixture of three independent samples (Zhang et al. 2012). All samples were transported on ice to the laboratory and filtered through 0.22 μm mixed cellulose esters membranes (47 mm diameter, Millipore, USA). The filter membranes containing the bacterial filtrates were stored at −80 °C until DNA extraction.

Water temperature and pH were measured by a HQd potable multi-parameters water quality analyzer (HACH, USA) in situ. Turbidity and total organic carbon (TOC) were determined using a 2100 AN turbidity analyzer (HACH, USA) and a 5310C TOC analyzer (GE, USA), respectively. Total phosphorus, ammonia nitrogen and chemical oxygen demand (COD) (determined by the
permanganate method, COD$_{Mn}$) were analyzed according to standard protocols (APHA 2011).

**Molecular analyses**

DNA was extracted using the cetyl trimethyl ammonium bromide (CTAB) method (Shan et al. 2011), and then the purity and concentration of DNA were determined by agarose gel electrophoresis. Appropriate samples were placed in a centrifuge tube and diluted with sterile water to 1 ng/µL. Using the diluted genomic DNA as the template, specific primers with Barcode, Phusion® high-fidelity polymerase chain reaction (PCR) Master Mix with GC Buffer from New England Biolabs, and high-efficiency high-fidelity enzyme were used for the PCR according to the selection of sequencing region to ensure the efficiency and accuracy of amplification. The primers used to identify bacterial diversity were those in the 16S V4 region (515F and 806R). In addition, PCR products were determined by 2% agarose gel electrophoresis. According to the concentration of PCR products, the samples were mixed with equal amount. After the samples were thoroughly mixed, the PCR products were detected by 2% agarose gel electrophoresis, and the products were recovered by the gel recovery kit provided by Qiagen. Lastly, the TruSeq® DNA PCR-free Sample Preparation Kit was used for library construction. The constructed library was quantified by Qubit and Q-PCR. After the library was qualified, HiSeq2500 PE250 was used for computer sequencing.

**Sequence processing and data analysis**

According to Barcode sequence and PCR amplified primer sequence, the sample data were separated from the computer data. After cutting Barcode sequence and primer sequence, FLASH was used to splice reads of each sample (Magoč & Salzberg 2011), and the resulting splicing sequences were the original Tags data (Raw Tags). Raw Tags were filtered strictly to obtain Clean Tags (Bokulich et al. 2015). The Tags obtained after the above processing were processed to remove chimera sequences. The chimera sequences were checked by comparing with the database, and finally removed to obtain the final Effective Tags (Haas et al. 2011). UPARSE software (Edgar 2013) was used to cluster Effective Tags from all samples. By default, the sequences were clustered into operational taxonomic units (OTU) with 97% identity. QIIME software (Caporaso et al. 2010) was used to calculate the alpha diversity indices including Observed species, Chao1, Shannon, Simpson, ACE, Goods-coverage.

Pearson’s correlation analysis of bacterial communities with the water characteristics was carried out using SPSS 19.0 software.

**RESULTS**

**Bacterial community diversity**

In this study, based on 16S rRNA sequencing analysis by Illumina HiSeq sequencing, bacterial community diversity in the three cascade reservoirs was investigated. The rarefaction curves of all water samples almost reached a plateau (Figure S1), indicating that these water samples were well analyzed. The OTUs and alpha diversity indices, consisting of Observed species number, Shannon index, Simpson index, Chao1 estimator and ACE estimator of the three reservoirs in the wet and dry seasons are shown in Table S2. The numbers of OTUs in YT, SZ and SY water sample groups were 1193–2622, 952–2950 and 959–3062, respectively. The values of Shannon index, one of community diversity indices, of YT, SZ and SY were 5.796–8.570, 4.795–8.915 and 5.515–8.875. Additionally, the values of Chao1 estimator, one of community richness indices, of YT, SZ and SY were 1153.140–2537.521, 930.058–2925.198 and 945.709–2967.793. On average, the OTUs and all alpha diversity indices of the water samples collected in the wet season were higher than in the dry season. Furthermore, as shown in Figure 2, the means for these parameters of the three water sample groups followed a spatial trend in the order of YT > SZ > SY. All the results indicated spatiotemporal variation occurred in the three cascade reservoirs.

**Bacterial community composition**

In this study, 20–34 bacterial phyla, 43–76 bacterial classes, 80–142 bacterial orders, 140–253 bacterial families and
179–419 bacterial genera were identified in the 36 water samples (Table S3). Figure 3 shows the relative abundances of the top 15 phyla in the water samples. *Proteobacteria*, *Cyanobacteria*, *Actinobacteria*, *Bacteroidetes* and *Firmicutes* were the main bacterial phyla, accounting for 82.59–97.07%.

*Proteobacteria*, accounting for 41.33% averagely, was the most dominant bacterial phylum. Notably, its average relative abundances in the three reservoirs in the wet season (47.51% of YT, 45.19% of SZ and 47.27% of SY) were higher than those in the dry season (41.60% of YT, 36.29% of SZ and 30.68% of SY), suggesting an apparent temporal variation. *Proteobacteria* was primarily composed of *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria* and *Epsilonproteobacteria* (Figure 4). Among the five subclasses, *Betaproteobacteria* was the most abundant one, with average relative abundances of 22.67% (YT), 19.61% (SZ), and 16.29% (SY). The average relative abundances of other bacterial classes from large to small were *Gammaproteobacteria* (10.99%), *Alphaproteobacteria* (7.78%), *Deltaproteobacteria* (2.10%) and *Epsilonproteobacteria* (0.28%). Notably, *Cyanobacteria* became predominated in March, and its relative abundances of YT3, SZ3 and SY3 were 45.39%, 56.70% and 53.97%.
A large variation in the composition of bacterial community in the water samples was observed at the genus level (Figure 5). The hgcI_clade, belonging to Actinobacteria, had a relatively high proportion in most water samples. Gammaproteobacteria, Stenotrophomonas and Acinetobacter were present in water samples YT5 and SY4, while Acinetobacter was also abundant in water sample SY5. Dechloromonas, a member of Betaproteobacteria, only predominated in water sample YT2. In addition, Sphingomonas, which is affiliated to Alphaproteobacteria, was numerous in water sample SZ5. Chryseobacterium and Sphingobacterium pertain to Bacteroidetes were plentiful in water sample SY3, and Chryseobacterium was also in a relatively high proportion in water sample SY4. Acinetobacter, Sphingomonas, Sphingobacterium and Pseudomonas are resistant to chlorine (Sun et al. 2011), and their relatively high abundance may create difficulty in achieving drinking water safety criterion. Furthermore, it is noted that Acinetobacter, Pseudomonas and Arcobacter are potential pathogenic bacteria.

**Potential pathogenic bacteria**

In order to further assess the potential pathogenic risk in the three cascade reservoirs, the potential pathogenic bacteria were analyzed by comparing the genera with the known potential pathogenic genera (WHO 2011; Ye & Zhang 2011). Twenty potential pathogenic genera were found (Table S4, Table S5 and Table S6). Although potential pathogenic genera were in low abundances, most of them prevailed in all the water samples. Acinetobacter, Arcobacter, Mycobacterium, Pseudomonas and Staphylococcus, whose relative abundances were more than 1% in at least one sample, were the main potential pathogenic genera. Besides Acinetobacter and Pseudomonas, Mycobacterium is also resistant to chlorine (Simões & Simões 2013; Lin et al. 2014). In particular, the average relative abundances of potential pathogenic genera detected in the wet season (6.89% of YT, 5.65% of SZ and 10.69% of SY) were higher than those in the dry season (4.34% of YT, 3.10% of SZ and 2.54% of SY) (Figure S2). The average relative abundances of potential pathogenic genera detected in the three cascade reservoirs followed an apparent spatial trend in the order of SY (6.62%) > YT (5.61%) > SZ (4.37%) (Figure S3).

**Relationship between the bacterial community and water characteristics**

SPSS 19.0 software was used to analyze Pearson’s correlation of the bacterial community indices, the relative abundance of Proteobacteria and the sum of relative abundance of potential pathogenic genera with the water characteristics (shown in Table S7). As shown in Table 1, water temperature showed prominent positive correlation with OTUs ($P < 0.01$), Shannon ($P < 0.05$), Chao1 ($P < 0.01$), Proteobacteria ($P < 0.05$) and potential pathogenic genera ($P < 0.05$). There were also significant positive relationships between potential pathogenic genera and turbidity and COD$_{Mn}$ (both $P < 0.05$).
DISCUSSION

Temporal variations

The average bacterial community diversity and relative abundance of *Proteobacteria* and potential pathogenic genera showed obvious temporal variation, with a higher average diversity and relative abundance in the wet season compared to the dry season (Table S2, Figure 3 and Figure S2). Such temporal variation was thought related to the water temperature in the reservoirs because a higher temperature within a certain range is favourable for microbial growth. It has been reported that bacterial community composition experienced notable alternation with a shift of wet and dry seasons in subtropical water bodies (Araújo & Leal 2008; Yu et al. 2019). In the studied city, the wet season covers April to September, during which water temperature in the reservoirs was 27.56 ± 1.06 °C (Table S7). The dry season runs from October to March the following year, during which water temperature in the reservoirs was 21.61 ± 3.25 °C (Table S7). Detailed examination revealed that the values of OTUs, Shannon and Chao1, and the relative abundance of *Proteobacteria* and the sum of relative abundance of potential pathogenic genera correlated positively with water temperature (Table 1). In the practices of the drinking water plants with the three reservoirs as the water sources, sequential chlorination and chlorine dioxide disinfection have been used to deal with the risk of microbial outbreaks in the wet season (Qie & Zhang 2017).

The sum of relative abundance of potential pathogenic genera was also observed to be correlated with turbidity (Table 1). It might be because turbidity can provide a habitat and shielding for potential pathogenic bacteria. Previous studies have shown turbidity is closely related to potential pathogenic bacteria abundance in waters (Huey & Meyer 2010), and the abundances of particle-associated and freeliving microbial communities were reportedly proportional to turbidity (Dang & Lovell 2009). Turbidity is hence listed as a microbiological indicator in the *Safe Drinking Water Act* in United States (United States Environmental Protection Agency 2009). In the present study, the average turbidity in the wet season was higher than that in the dry season in the three reservoirs. This trend was consistent with the temporal variation of potential pathogenic genera. The requirement for turbidity in the *Standards for Drinking Water Quality* (Ministry of Health of the People’s Republic of China/Standardization Administration of China 2006) in China is no greater than 1.0 NTU (nephelometric turbidity unit). In the downstream drinking water plants of the three reservoirs, the turbidity in the finished water produced by conventional treatment process is already less than 0.3 NTU. However, higher total plate counts are often detected in the wet season compared to the dry season, although it can still meet the water quality standard. This indicates a higher microbial risk in the wet season. For conservative consideration, additional measures are suggested to ensure the safety of drinking water. Ozone biological activated carbon (O3–BAC) advanced treatment process was therefore supplemented in one drinking water plant. Long-term

| Table 1 | Pearson’s correlation coefficients describing the relationship between water characteristics and the bacterial community indices, the relative abundance of *Proteobacteria* and the sum of relative abundance of potential pathogenic genera |
|---------|--------------------------------------------------------------------------------------------------|
| Water characteristics | OTUs | Shannon | Chao1 | Proteobacteria | Potential pathogenic genera |
| pH | 0.058 | −0.405 | 0.035 | −0.097 | 0.054 |
| Water temperature | 0.479** | 0.393* | 0.451** | 0.336* | 0.345* |
| Turbidity | 0.035 | 0.080 | 0.271 | 0.270 | 0.421* |
| Total phosphorus | −0.022 | 0.061 | −0.009 | −0.187 | −0.037 |
| Ammonia nitrogen | 0.159 | 0.060 | 0.122 | 0.241 | 0.133 |
| TOC | 0.268 | 0.056 | 0.205 | 0.144 | 0.236 |
| CODMn | 0.113 | −0.008 | 0.083 | 0.216 | 0.400* |

**There is a significant correlation at the 0.05 level (bilateral).**

**There is a remarkable correlation at the 0.01 level (bilateral).**
operation showed that the turbidity in the finished water remained below 0.1 NTU, and the total plate count was almost undetected (no more than 10 colony forming units/mL) (Zhang & Liu 2017).

Spatial variations

For the bacterial community composition, Proteobacteria was the most dominant bacterial phylum in most of the water samples collected from the three reservoirs (Figure 3). This is a common observation in many drinking water sources, such as reservoir water (Yu et al. 2014; Li et al. 2017), lake water (Ji et al. 2019), river water (Fiedler et al. 2018), spring water (Leon et al. 2013) and groundwater (Chen et al. 2019). The bacterial community diversity followed the order of YT > SZ > SY (Figure 2), which was consistent with the water current direction. A similar observation was reported in a series of six cascade reservoirs in Brazil where the cell density of total bacterioplankton decreased with the water current (Abe et al. 2005).

Nevertheless, the average relative abundances of potential pathogenic genera followed an apparent spatial trend in the order of SY > YT > SZ (Figure S3). This trend might be related to the degree of water pollution in the three reservoirs. The detected CODMn in the water samples also followed a similar trend: SY (3.36 ± 0.74) > YT (2.70 ± 0.62) > SZ (1.75 ± 0.44) (Table S7). Reservoir YT, as a diversion reservoir, receives water from the Dongjiang River, whose water quality conformed to the requirement of Class II of China’s national surface water quality standard. It was the first-level water source protection zone and can be used as a habitat for rare aquatic life (MEE 2019). Reservoir SZ is a very important drinking water source, and supplies raw water not only to local water treatment plants but also to Hong Kong. Thus, a sidestream biological treatment facility (4,000,000 m³/d) was implemented in the reservoir to obtain better water quality. This might have resulted in the lower relative abundance of potential pathogenic genera. However, the area surrounding reservoir SY is not well developed, it includes many industrial zones, and non-point source pollution was not well controlled due to the lack of pollution mitigation measures for outside discharge. Its water quality conformed to the requirement of Class III of China’s national surface water quality standard. It was the second-level water source protection zone with some aquaculture zones. These may lead to the higher relative abundance of potential pathogenic genera. During the fast development of the protection measures in the surrounding area, enhanced chlorination measures, such as prechlorination and multi-point chlorination, have been used in drinking water treatment plants where reservoir SY is the water source for ensuring drinking water safety. It ensures the total plate count in finished water meets the requirement of the Standards for Drinking Water Quality in China.

Potential pathogenic bacteria

Potential pathogenic bacteria were analyzed in detail to assess whether control measures in the drinking treatment plants were adequate and whether more safeguards should be taken to guarantee drinking water safety. In this study, even though not in high abundances, 20 potential pathogenic genera were detected in the three reservoirs (Table S4, Table S5 and Table S6). The main potential pathogenic genera included Acinetobacter, Arcobacter, Mycobacterium, Pseudomonas and Staphylococcus, and Acinetobacter, Mycobacterium and Pseudomonas were resistant to chlorine (Sun et al. 2017). Studies have reported that 22 and 76 potential pathogenic genera were detected in the Yangtze River in Jiangsu Province (Sun et al. 2017) and the Liushahe River in Yunnan Province (Liu et al. 2018a), respectively. This suggests the number of potential pathogenic genera detected in the three cascade reservoirs was almost the same as or lower than other water sources, and the risks of potential pathogenic genera were not significant compared to other water sources. Although chlorination can inactivate most potential pathogenic bacteria, there were still some potential pathogenic genera detected in the finished water and pipe water due to their chlorine resistance. For example, Acinetobacter and Mycobacterium were found in the finished water of a drinking water plant using conventional treatment process (Lin et al. 2014), and Mycobacterium was determined in a distribution system (Chen et al. 2012). In addition to the strong resistance of Mycobacterium to chlorine, Mycobacterium could cause biofilm formation (Le Dantec et al. 2002). As the supplementary measures of conventional treatment process, ozonation...
(Zhang et al. 2013) and ultraviolet disinfection (Sun et al. 2013) have demonstrated their effectiveness in killing potential pathogenic bacteria, especially those resistant to chlorine. In some drinking water treatment plants where the three reservoirs are the water sources, ozonation and ultraviolet disinfection have successfully been proven to reduce potential pathogenic bacteria.

**CONCLUSIONS**

A large spatiotemporal variation in diversity and structure of bacterial communities was observed in the three cascade reservoirs over a whole hydrological year. Proteobacteria (especially Betaproteobacteria) predominated in most water samples, and five genera resistant to chlorine (Mycobacterium, Acinetobacter, Sphingomonas, Sphingobacterium and Pseudomonas) and 20 potential pathogenic genera (mainly Acinetobacter, Arcobacter, Mycobacterium, Pseudomonas and Staphylococcus) were detected. The bacterial community diversity and the average relative abundance of potential pathogenic genera were higher in the wet season than in the dry season, and water temperature was the major factor leading to the temporal variation. They also showed distinct spatial variations in the three cascade reservoirs. The spatial variation of bacterial community diversity was found to be related to water current, and the potential pathogenic genera was influenced by the degree of water pollution. Water turbidity and COD \(_{\text{Mn}}\) found to be the dominant factors relating to the average relative abundance of potential pathogenic genera.

In order to secure the bacterial safety of drinking water, necessary measures, such as the removal of turbidity and COD \(_{\text{Mn}}\), should be carried out as soon as possible. There should also be further studies on inactivation of potential pathogenic bacteria.

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**CONFLICTS OF INTEREST**

The authors declare no conflict of interest. The authors are responsible for the content and writing of the paper.

**DATA AVAILABILITY STATEMENT**

All relevant data are included in the paper or its Supplementary Information.

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