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The drinking water treatment process as a potential source of affecting the bacterial antibiotic resistance

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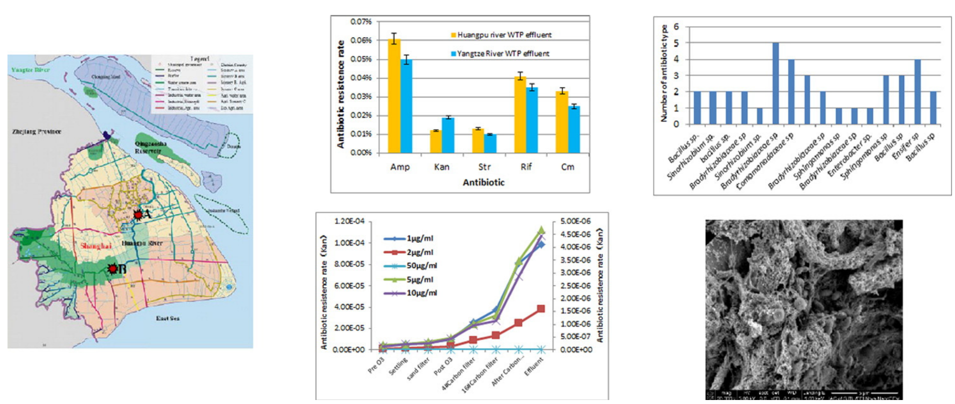
HIGHLIGHTS

- Bacterial antibiotic resistance rate increased as the water treatment progressed.
- Carbon filtration plays a key role in enhancing bacterial antibiotic resistance rate.
- Multidrug resistant bacteria were isolated and identified in processed water.
- Ozone, BAC and disinfection can greatly affect the community abundance.

GRAPHICAL ABSTRACT



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ABSTRACT

Two waterworks, with source water derived from the Huangpu or Yangtze River in Shanghai, were investigated, and the effluents were plate-screened for antibiotic-resistant bacteria (ARB) using five antibiotics: ampicillin (AMP), kanamycin (KAN), rifampicin (RFP), chloramphenicol (CM) and streptomycin (STR). The influence of water treatment procedures on the bacterial antibiotic resistance rate and the changes that bacteria underwent when exposed to the five antibiotics at concentration levels ranging from 1 to 100 μg/mL were studied. Multidrug resistance was also analyzed using drug sensitivity tests. The results indicated that bacteria derived from water treatment plant effluent that used the Huangpu River rather than the Yangtze River as source water exhibited higher antibiotic resistance rates against AMP, STR, RFP and CM but lower antibiotic resistance rates against KAN. When the antibiotic concentration levels ranged from 1 to 10 μg/mL, the antibiotic resistance rates of the bacteria in the water increased as water treatment progressed. Biological activated carbon (BAC) filtration played a key role in increasing the antibiotic resistance rate of bacteria. Chloramine disinfection can enhance antibiotic resistance. Among the isolated ARB, 75% were resistant to multiple antibiotics. Ozone oxidation, BAC filtration and chloramine disinfection can greatly affect the relative abundance of bacteria in the community.

1. Introduction

The emergence of bacterial antibiotic resistance is common in areas where antibiotics are used (Julian and Dorothy, 2010). The widespread use of antibiotics in medicine, intensive animal husbandry, industrial

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settings and their release in wastewater treatment plants are main sources of the selective pressure exerted on bacteria (Marti et al., 2013, 2014; Schwartz et al., 2003; Sheetal et al., 2013). Because antibiotic selection pressures are so prevalent (Kemper, 2008; Kümmerer, 2003), the antibiotic resistance of pathogenic bacteria represents a global health problem that requires a better understanding of the fate of antibiotic-resistant bacteria (ARB) in water environments and their spread in the water supply system (Baquero et al., 2008; Huerta et al., 2013; Jiang et al., 2013; Ramanan et al., 2013; Timothy et al., 2011). The wide and excessive use of antibiotics has resulted in the pollution of several of the world's surface and ground water sources by antibiotics and antibiotic-resistance genes (ARGs) (Chigor et al., 2010; Machado and Bordalo, 2014; Martinez, 2009; Olusegun et al., 2009). The concentrations of tetracyclines (TCs), sulfa antibiotics, and chloromycetin (CAP) antibiotics in the Huangpu River, which supplies the city of Shanghai, China with some of its drinking water, are in the range of 0.44 to 2.69 $\mu g \cdot L^{-1}$, 0.97 to 1.96 $\mu g \cdot L^{-1}$ and 0.03 to 0.26 $\mu g \cdot L^{-1}$ respectively (Shen et al., 2012). In early March 2013, over 16,000 dead pigs were found in this river; the pigs had been dumped by farmers from neighboring Jiaxing, Zhejiang Province, a major pig-farming area upstream of Huangpu River where antibiotics were used very popular for livestock (Davison, 2013). Thus, the aquatic ecosystem has been considered to be a critical source of ARB and ARGs (Tao et al., 2010; Zhang et al., 2009). The current water treatment process cannot entirely eliminate the existing antibiotics in the drinking water (Figueira et al., 2011; Guo et al., 2014; Laroche et al., 2010; Xi et al., 2009). Moreover, horizontal transmission of ARGs among microbes in the water supply system could facilitate the emergence and dissemination of bacterial antibiotic resistance in drinking water and influence human health (Ribeiro et al., 2014). However, there have been no systematic investigations examining the influence of different water treatment processes on bacterial antibiotic resistance, particularly in high-microbe-density environments, including biological activated carbon (BAC) and during oxidizing processes such as ozone oxidation and chloramine disinfection. These processes are crucial for the control of microbiological risk in drinking water.

Shanghai is the largest international metropolis in China, and Huangpu River is the primary source of drinking water in Shanghai. However, because of water pollution in the Huangpu River, the Qingcaosha Reservoir, which is the largest estuary and river reservoir in the world and which draws its water from the Yangtze River, has replaced a large portion of Shanghai's water source. This reservoir is located at the estuary, where various pollutants including certain antibiotics, may accumulate from the upstream cities' discharge. These two water sources are both facing antibiotic and ARG pollution and deserve more attention as a public health concern.

The current study compared the antibiotic resistance rates of bacteria in effluents from Shanghai waterworks using two main water sources and investigated the influence of water treatment procedures on bacterial antibiotic resistance using the following five antibiotics: ampicillin (AMP), kanamycin (KAN), rifampicin (RFP), chloramphenicol (CM), and streptomycin (STR). The multidrug resistance of isolated ARB was tested and the influence of the water treatment process on the environmental water metagenome was also analyzed using 16S rDNA high-throughput sequencing.

2. Materials and methods

2.1. Water sampling

As Fig. 1 showed, all water samples were taken from the effluent of each treatment process unit in two waterworks (A and B) that respectively used the Qingcaosha Estuary Reservoir or the Huangpu River for source water in 2013 spring and summer (March to August). All the samplings were under the help of the waterworks' engineers. The samples were taken once a month. All the sampling procedures met the standard examination methods for drinking water-collection and preservation of water samples (GB/T 5750.2-2006). 10 L water was collected at each site for antibiotic resistance bacteria isolation and DNA extraction. All water samples were stored in 2.5 L pre-cleaned and sterilized glass bottles, maintained at ice boxes and transported to the laboratory at once by a car. The water treatment process involved pre-ozonization, coagulation and sedimentation, sand filtration, post-ozonization, BAC filtration and chloramine disinfection, as shown in Fig. 2. In the BAC unit, two different types of activated carbon were applied: broken activated carbon particles used in 16# carbon filter and broken activated carbon columns in 4# carbon filter. We set 8 sampling points along the process as introduced above.

2.2. Antibiotics selection

Five popular used antibiotics in human health treatment and animal farms (AMP, KAN, RFP, CM and STR) were selected for this study. AMP is broad-spectrum semisynthetic penicillin that has low toxicity. The antibacterial spectrum of AMP is similar to that of penicillin. *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Proteus*, *Mycobacterium tuberculosis* and *Staphylococcus aureus* are sensitive to KAN, whereas *Pseudomonas aeruginosa*, Gram-positive bacteria (except *S. aureus*), anaerobes, atypical mycobacteria, *Rickettsia*, fungi and viruses are resistant to KAN. RFP can effectively kill *M. tuberculosis* and several of the nontuberculous mycobacteria inside and outside host cells. CM can inhibit the growth of Gram-positive and Gram-negative bacteria, and the inhibitory effect on Gram-negative bacteria is comparatively substantial. STR exerts a strong antibacterial effect on *M. tuberculosis*; conversely, nontuberculous mycobacteria are resistant to STR. There are many animal farms in the upstream watershed of Huangpu River, and the antibiotics like CM and STR were popular to be used for animal breeding. The bacteria affected by these five antibiotics include the primary bacteria that exist in drinking water.

2.3. ARB isolation and calculation of the antibiotic resistance rate

The steps for screening for ARB were as follows, using AMP as an example:

(a) An antibiotic solution was prepared by dissolving 50 mg of AMP in 10 mL of water; the concentration of the solution was thus 5 mg/mL. The colorless, transparent solution was then distributed into 10 tubes (1 mL per tube).
(b) A 1-L volume of R2A agar medium was prepared. The medium was heat sterilized under 121 °C and 15 min and cooled to a moderately warm temperature. AMP was then added to the medium, and thoroughly mixed. The medium was poured into plates and stored at room temperature overnight.
(c) A water sample extracted from the water treatment plant was filtered using a 0.22-μm filter membrane, which was then cut into pieces and placed in a 2-mL microcentrifuge tube. Then 2 mL of a phosphate buffer solution was added to the microcentrifuge tube, which was subsequently vortexed for 5 min at high speed.
(d) A general R2A medium-coated plate without antibiotics was employed for comparison with the medium-coated plate containing 5 μg/mL antibiotics. The later plate was inoculated and spread with the vortexed water sample, followed by incubation for 7 days at 28 °C. Each sample for ARB isolation at each antibiotic concentration level was repeated three times.
(e) The antibiotic resistance rate was calculated as follows.

Based on the aforementioned steps, ARB were counted and isolated on the medium-coated plates containing antibiotics. The antibiotic