Environmental Pollution 234 (2018) 71–78

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Sulfadiazine/ciprofloxacin promote opportunistic pathogens occurrence in bulk water of drinking water distribution systems☆

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ARTICLE INFO

Article history:
Received 26 September 2017
Received in revised form
6 November 2017
Accepted 11 November 2017
Available online 21 November 2017

Keywords:
Sulfadiazine
Ciprofloxacin
Opportunistic pathogens
Antibiotic resistance genes
Extracellular polymeric substances

ABSTRACT

Effects of sulfadiazine and ciprofloxacin on the occurrence of free-living and particle-associated opportunistic pathogens in bulk water of simulated drinking water distribution systems (DWDSs) were investigated. It was found that sulfadiazine and ciprofloxacin greatly promoted the occurrence of opportunistic pathogens including *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Mycobacterium avium* and its broader genus *Mycobacterium* spp., as well as the amoebae *Acanthamoeba* spp. and *Hartmanella vermiformis*, in bulk water of DWDSs. Moreover, sulfadiazine and ciprofloxacin exhibited much stronger combined effects on the increase of these opportunistic pathogens. Based on the analysis of the antibiotic resistance genes (ARGs) and extracellular polymeric substances (EPS), it was verified that EPS production was increased by the antibiotic resistant bacteria arising from the effects of sulfadiazine/ciprofloxacin. The combined effects of sulfadiazine and ciprofloxacin induced the greatest increase of EPS production in DWDSs. Furthermore, the increased EPS with higher contents of proteins and secondary structure β-sheet led to greater bacterial aggregation and adsorption. Meanwhile, large numbers of suspended particles were formed, increasing the chlorine-resistance capability, which was responsible for the enhancement of the particle-associated opportunistic pathogens in bulk water of DWDSs with sulfadiazine/ciprofloxacin. Therefore, sulfadiazine and ciprofloxacin promoted the occurrence of particle-associated opportunistic pathogens in bulk water of DWDSs due to the role of EPS produced by the bacteria with ARGs.

1. Introduction

In recent years, opportunistic pathogens in drinking water have become an emerging public health concern (Wang et al., 2013). Opportunistic pathogens including *Legionella pneumophila*, *Mycobacterium avium*, *Pseudomonas aeruginosa*, and the amoeba *Acanthamoeba* spp., have sometimes been found in drinking water distribution systems (DWDSs) and tap water, which can cause disease in individual, especially immunocompromised populations (Wang et al., 2012a, 2012b).

Opportunistic pathogens naturally colonize, persist and multiply in DWDSs (Wang et al., 2013). They can establish as a part of the microbial ecology in DWDSs, and the interactions with other bacteria can stimulate their growth (Declerck et al., 2009; Wang et al., 2013). Intracellular replication within free-living amoebae host, such as *Acanthamoeba* spp., is thought to be an important reason for *Legionella* and *Mycobacteria* growth in DWDSs (Delafont et al., 2014; Thomas et al., 2014). Biofilms are prevalent in DWDSs and can also impact opportunistic pathogens growth, which is due to ecological interactions including competition, antagonism and symbiosis between the biofilms bacterial community and the opportunistic pathogens (Wang et al., 2014; Miller et al., 2015).

Bacteria in bulk water are usually separated into free-living and particle-associated bacteria (D'Ambrosio et al., 2014). The particle-associated bacteria are formed due to bacterial attachment onto the suspended particles in the water (Winkelmann and Harder, 2009).

☆ This paper has been recommended for acceptance by Klaus Kummerer.

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https://doi.org/10.1016/j.envpol.2017.11.050
0269-7491/

The particle-associated bacteria are often larger and more resistant to disinfection by chlorine, ozone and ultraviolet (UV) radiation than free-living bacteria (Dietrich et al., 2007; Kollu and Ormeci, 2012). Because of ineffective removal of suspended particles in drinking water treatment processes, many particle-associated opportunistic pathogens are also found in DWDSs (Liu et al., 2013a). Therefore, the suspended particles are the important factors affecting the occurrence of opportunistic pathogens in DWDSs. However, there is little known about the effects of source water quality on the formation of particle-associated opportunistic pathogens in DWDSs.

Many antibiotics, including sulfonamides and quinolones, are detected in surface water at the level of μg L^{-1} or ng L^{-1} (Johnson et al., 2015). Sulfadiazine and ciprofloxacin are representative sulfonamide and quinolone antibiotics, respectively. Because these antibiotics cannot be removed effectively through conventional treatment processes, trace levels of these antibiotics have been detected in finished drinking water and tap water (Ye and Weinberg, 2007; Han et al., 2010; Jia et al., 2015). After long-term exposure to the antibiotics, changes of the microbial community and the promotion of antibiotic resistance genes (ARGs) in water have been observed (Harnisz, 2013; Tandukar et al., 2013; Ory et al., 2016). There is a strong correlation between bacterial community shift and ARGs alteration (Jia et al., 2015). However, to date, few studies on the relationship between ARGs and opportunistic pathogens have been conducted in DWDSs.

Moreover, one study has found a significant increase in extracellular polymeric substances (EPS) production when active cells of *Rhodococcus jostii* strain RHA1 were exposed to pollutants such as perfluoroalkyl acids (Weathers et al., 2015). EPS can provide a protective barrier to the bacteria, which increase the bacterial resistance to disinfectants. The mechanisms of EPS protection for bacteria include transport limitation of disinfectants through the EPS matrix and sacrificial reaction of EPS with disinfectants (Xue et al., 2014). EPS with different composition and structure can also affect bacterial aggregation and adsorption onto particles (Adeleye and Keller, 2016; Jia et al., 2017; Lin et al., 2017). However, there are few studies focused on the EPS production and composition changes in DWDSs in the presence of antibiotics, and few reports about the influence of EPS compositions on particle-associated opportunistic pathogens formation in bulk water of DWDSs.

Therefore, the objectives of this study are (1) to investigate the occurrence of free-living and particle-associated opportunistic pathogens in bulk water of DWDSs with sulfadiazine/ciprofloxacin, and (2) to elucidate the effects mechanism of these antibiotics on the occurrence of opportunistic pathogens by analyzing the roles of ARGs and EPS.

2. Materials and methods

2.1. Materials

Sulfadiazine and ciprofloxacin, high performance liquid chromatography grade, were purchased from Sigma-Aldrich Fluka (USA). Sodium hypochlorite solution, analytical grade, was obtained from Sinopharm Chemical Reagent Co., Ltd (China).

The tested raw water was collected from a drinking water treatment plant in north of China, which was treated with coagulation using polyaluminium chloride, sedimentation, sand filtration, and biologically-activated carbon filtration (prior to entering the chlorine contact tanks). Water quality parameters were measured according to standard methods (EPA of China, 2002), and the results were shown in Supporting Information (Table S1). Differences of water quality were assessed using analysis of variance (ANOVA) with a significance threshold of $\alpha = 0.05$.

2.2. Experimental set-up and operation

Ten cast iron coupons were immersed in covered 1.5 L glass fiber-reinforced plastic bottles to simulate the DWDSs. The characteristics of the cast iron coupons were described in Supporting Information (Text S1). Before this study, twelve simulated DWDSs have been run under the same conditions for more than three years, and stable corrosion scales have been formed on the surface of cast iron coupons. Four kinds of waters, including raw water, raw water with addition of 2 μg L^{-1} sulfadiazine, raw water with addition of 2 μg L^{-1} ciprofloxacin, and raw water with addition of 1 μg L^{-1} sulfadiazine and 1 μg L^{-1} ciprofloxacin, were used in this study. After 1 L of test water was chlorinated for 2 h with 1 mg L^{-1} chlorine (NaClO solution), the four kinds of waters were poured into the DWDSs, respectively. Each experiment was done in triplicate. The total chlorine concentration was measured using a HANNA spectrophotometer (HI93711, Italy).

The water in each DWDS was displaced with fresh water at 48 h intervals and gently agitated by a magnetic rotor to mix the water, reflecting dead zones or worst case conditions in actual water distribution systems according to previously reported methods (Liu et al., 2013b; Wang et al., 2014). Addition of sulfadiazine and ciprofloxacin to the raw water changed the bacterial community and water quality in effluents of the DWDSs. According to other studies and our previous studies (Wang et al., 2012b, 2017a), water quality can reach a relatively stable value after 8 months. Therefore, after 8 months, the 16S rRNA gene, ARGs, EPS, and the free-living and particle-associated opportunistic pathogens in the effluents of DWDSs were analyzed. The counts of particles with different sizes were also measured by a particle counter (GR-1500A, Lvjie, China).

2.3. Antibiotics analytical methods

When the sulfadiazine and ciprofloxacin were added to the raw water, the antibiotics in the used water, influents (chlorinated water) and effluents of the DWDSs were concentrated by solid phase extraction (SPE) method using an HLB cartridge (200 mg/6 mL) (Waters Oasis). Then, the exactly concentrations of the antibiotics were determined by an Ultra Performance Liquid Chromatography-Tandem Mass Spectrometer (UPLC-MS/MS, Quattro Premier XE, Waters, USA). Sulfadiazine and ciprofloxacin were not detected in the raw water. The detailed operation methods were listed in Text S2.

2.4. Sample collection, PMA treatment and DNA extraction

1 L bulk water of DWDSs effluents was filtered through 1.2 μm and 0.2 μm polycarbonate filters using a sterile filter funnel and vacuum flask setup, sequentially. Filters were stored in sterile 2 mL microfuge tubes before DNA extraction. According to other studies, the bacteria in 1.2 μm and 0.2 μm polycarbonate filter were regarded as particle-associated and free-living bacteria, respectively (Zhang et al., 2007; Liu et al., 2013a).

The propidium monoazide (PMA)-bound DNA cannot be amplified in the subsequent PCR. This characteristic is often applied to quantify the DNA of live bacteria and characterize the changes in viable bacterial communities (Gensberger et al., 2014). The process of PMA treatment of samples was described in Text S3. An optimum PMA concentration of 40 μM was determined after testing a range of concentrations to maximize removal of DNA from 70%-isopropanol-killed *Escherichia coli* cells, while minimizing DNA removal from live cells (Supporting Information, Fig. S1). This optimum PMA concentration of 40 μM was validated on untreated