Risk Assessment and effect of Penicillin-G on bacterial diversity in drinking water

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Abstract: Penicillin-G was detected in drinking water by LC-MS/MS and the bacterial diversity was investigated by PCR and high-throughput sequencing. The results showed that bacteria community structure in drinking water has undergone major changes when added different concentrations of penicillin-G. The diversity index of each sample was calculated. The results showed that the total number and abundance of bacterial community species in drinking water samples decreased significantly after the addition of penicillin-G. However, the number and abundance of community structure did not change with the concentration. Penicillin-G inhibits the activity of bacterial community in drinking water and can reduce the bacterial diversity in drinking water.

1. Introduction

As a kind of new pollutant, pharmaceutical and personal care products (PPCPs) are long-lasting, traceable and biodegradable pollutants in the environment [Lindstrom A., et al., 2002], and are closely related to human life. PPCPs including a wide range of chemical substances, human and veterinary drugs and other chemicals consumer products, such as anti-inflammatory drugs, sedatives, antibiotics, cosmetics, musk substances, sunscreens, hair dyes, hair sprays, soaps, shampoos and so on[Bound J.P., et al., 2006]. In recent years, there has been a steady increase in the number of systematic studies concerning the occurrence of PPCPs especially in aquatic environment [Schreurs R.H., et al., 2005].

Although the concentration of PPCPs is very low, they have strong biological activity. Due to their molecular characters and chemical structure, the rate of PPCPs biodegradation were very slow. Most of them have effects of carcinogenic, teratogenic and mutagenic [Wen et al., 2013]. PPCPs follow different pathways in the environment and they are massively transported in Wastewater Treatments Plants (WWTPs), which have been pointed out as the primary sources of these contaminants' pollution into the water bodies [Myrsini P. et al., 2016]. With the effluent of the WWTP entering the receiving water bodies, PPCPs pose a potential threat to groundwater and surface water, which in turn affects the drinking water safety.

Until now, several researchers from all over the world have focused on the occurrence of PPCPs in different environmental matrices such as surface water, groundwater, waste water and even drinking water systems [McArdell C., et al., 2003; Farré M., et al., 2005; Constanzo S.D., et al., 2005; Xu W.H., et al., 2007; Sacher F., et al., 2001; Watkinson A.J., et al., 2009; Yiruhan, Q.J., et al., 2010]. There are many studies on PPCPs in drinking water systems. Ternes found clofibrate in tap water at levels ranging from a few ng/L to 70 ng/L, while sedatives were also detected in some tap water samples [Ternes T.A., 1998]. Heberer reported that more than 80 drugs and their metabolites have been detected in sewage, surface water and groundwater of 12 countries, and trace amounts of several drugs have been detected in drinking water [Heberer T., 2002]. Loraine et al detected seven kinds of PPCPs at four treatment

plants in southern California [Loraine G.A., et al., 2006]. In addition, Yu's results showed that 12 kinds of PPCPs were monitored in water plant and 5 kinds of PPCPs were monitored in the distribution network [Yu et al., 2010].

Penicillins and cephalosporins are beta-lactam antibiotics, which can prevent the synthesis of bacterial cell wall and thus inhibit bacterial growth. These kinds of antibiotics have become the world's largest and most widely used antibiotic. Some antibiotics discharged without biodegradation, which result in the large amount of beta-lactam antibiotics are released into the environment. The result of our research group showed that penicillin-G is a kind of PPCPs with high detection frequency and high concentration in the distration network [Zhang, 2014]. In this paper the interaction between penicillin-G and bacteria diversity in drinking water were investigated, and the bacterial risk was assessed.

2. Materials and methods

2.1. Water samples
Water samples were taken from drinking water distribution networks and residual chlorine was eliminated by adding ascorbic acid. The penicillin-G (purchased from TRC Corportation, Yorkshire, Canada) were added at the final concentration of 10 ng/L, 20 ng/L, 50 ng/L and 100 ng/L, and there was a comparison blank control. The blank control water sample was sterilized by high-pressure steam, and penicillin-G at different concentrations were added as other water samples. The experimental period was 20 days, and the water samples were collected in triplicate and analyzed immediately after collection. The pH value and dissolved oxygen (DO) concentration were immediately measured using a portable Hach DO/pH/Eh meter (Hach SensION+DO6). Free chlorine was measured by a Hach (PCII); and turbidity was measured by a Hach 2100N following their standard calibration and operational methods.

The water quality indicators of drinking water sample are shown in table 1.

Table 1 Water quality standards and the measured quality of the drinking water

Water quality parameters	Water quality standards	Drinking water quality
Turbidity (NTU)	1	0.6
pH	6.5≤pH≤8.5	6.62
Temperature (°C)	-	15
Free chlorine (mg/L)	0.05≤FC≤0.3	0.16
DO	-	8.01 mg/L
BDOC (mg/L)	-	0.23 g/L

2.2 Determination of penicillin-G
The samples were enriched by solid-phase extraction (SPE). The eluate was collected in a test tube and was evaporated using nitrogen sparging. The sample was reconstituted to a final volume of 1 mL with 10% methanol (v/v) and transferred to an amber auto sampler vial for LC-MS/MS analysis [Liu et al., 2016]. The chromatographic separation of the analyses was conducted using an ACQUITY Ultra Performance liquid chromatograph (UPLC) and the mass spectrometric measurements were performed on a Quattro Premier XE (Waters, USA) equipped with an electrospray ionization source. All samples were analyzed in duplicate to provide a 10% average coefficient of variation for the duplicated samples.

To investigate the effects of tube wall adsorption of penicillin-G in water samples, a tube wall adsorption experiment was performed, and the results showed that the effects of wall adsorption on penicillin-G was very small and could be ignored.

2.3 Bacterial diversity analysis
Total bacterial DNA in the water samples was extracted using water DNA Kits D5525-02 (Omega, USA) following the manufacturer's protocol. Extracted genomic DNA was detected by 1% agarose gel electrophoresis. The bacterial 16S rRNA (V3+V4) genes were amplified, and bacterial diversity in the samples was detected by Illumina HiSeq 2000.

3. Results and discussion

3.1 Concentration of penicillin-G
The concentrations of penicillin-G in blank control and water samples with penicillin-G added were detected by LC-MS/MS after 20 days, and the results were shown in table 2. There was no penicillin-G detected in the blank control.

Table 2 Concentrations of penicillin-G in drinking water samples (ng/L).

Added penicillin-G concentration	10	20	50	100
Blank control	9.3	17.7	45.5	93.2
Water samples with penicillin-G added	7.1	12.0	41.8	91
Value differences	2.2	5.7	3.7	2.2

As can be seen from Table 2, the concentration of penicillin-G in non-sterilized drinking water samples was lower than that in blank control samples. The result indicated that the presence of bacteria in drinking water has a certain effect on degradation of penicillin-G.

3.2 Bacterial diversity analysis

3.2.1 Bacteria diversity analysis of water sample without penicillin-G added   Total DNA was extracted from the drinking water samples that no penicillin-G added, and the bacterial diversity was analyzed. GWS2 in Figure 1 is the bacteria diversity at genus level of water samples without penicillin-G added. From the figure we can see that *unknown bacteria* accounted for the most proportion of bacteria, and the abundance is about 30%. The abundance of *Sphingopyxis* is about 12%; the abundance of *Sphingomonas* is about 10%; the abundance of *Rhodobacter* is about 8%; the abundance of *Hyphomicrobium* is about 7% and the abundance of *Porphyrobacter* is about 7%. The abundance of *Uncultured Brucells* and *Lactococcus* is about 4%; the abundance of *Phenylobacterium* is about 4% and other species such as *Blastomonas*, *Bradyrhizobium*, *Staphylococcus*, *Pseudomonas*, *uncultured bacterium* accounted for a relatively small proportion.

3.2.2 Bacterial diversity analysis of water sample   After different concentrations of penicillin-G were added and reacted for 20 days, total DNA of the water samples were extracted and bacterial diversity were analyzed. The results were analyzed on genus level (Figure 1). Q1 to Q4 represents penicillin-G at concentration of 10ng/L, 20ng/L, 50ng/L and 100ng/L, respectively. From Figure 1we can see bacteria abundance of each water samples.
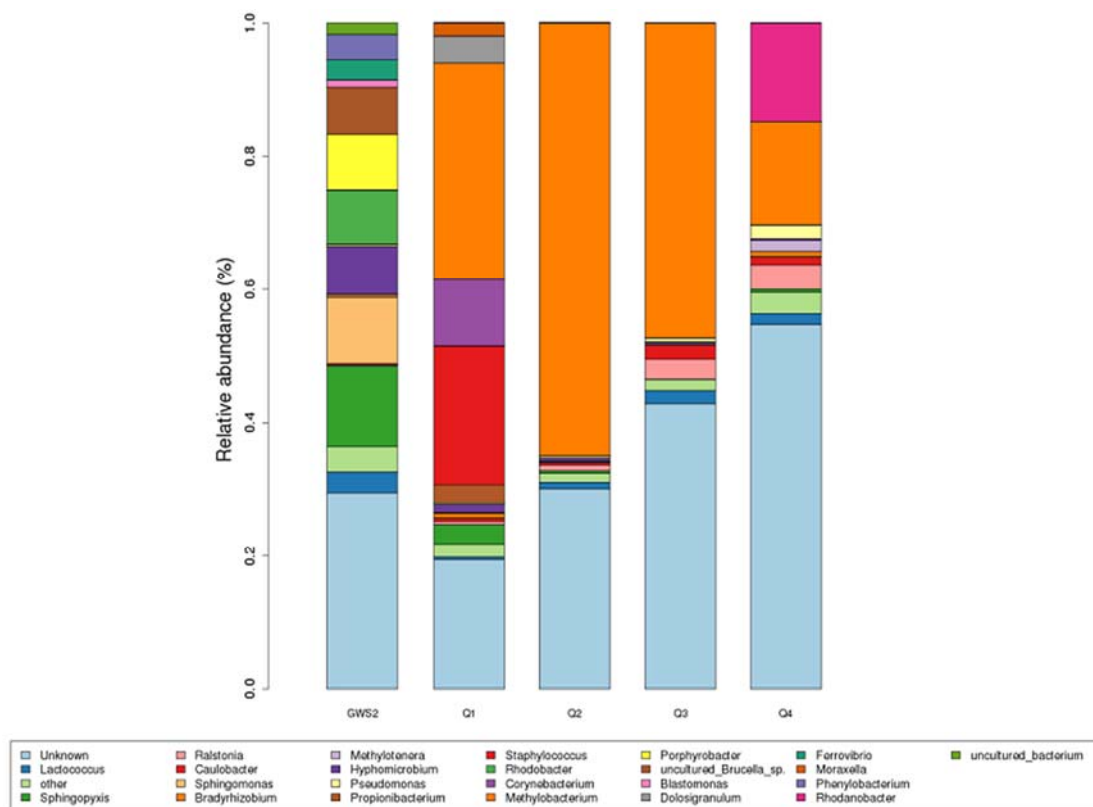
Figure 1. Bacterial structure in drinking water with different concentration of penicillin-G added.

After different concentrations of penicillin-G were added in drinking water samples, bacteria community structure changed greatly. Compared with water samples without penicillin G, the abundance of *unknown* species and *Lactococcus* decreased, but the concentration increased gradually with the increase of penicillin-G concentration. The abundance of *Lactococcus* reached to the lowest when the concentration of penicillin-G was about 10 ng/L, and the abundance of *unknown* species reached to 52% when the concentration of penicillin-G was about 100 ng/L. The abundance of *Sphingopyxis* is about 12% of the water samples without penicillin-G. The abundance decreased to 3% when penicillin-G concentration was 10ng/L, and then decreased gradually with the increase of penicillin-G concentration. The abundance of *Sphingomonas*, *Hyphomicrobium*, *Rhodobacter*, *Porphyrobacter*, *uncultured Brucells*, *Ferrovibrio*, *Phenylobacterium*, *uncultured bacterium* were higher in water samples without penicillin-G, but they nearly disappeared in water samples with penicillin-G added. The results indicated that these bacteria are more sensitive to penicillin-G and their biological activity are inhibited by penicillin-G.

There was no *Methylobacterium* detected in water samples without penicillin-G added, while the abundance of *Methylobacterium* in the water samples with different concentrations of penicillin-G added was 15%-64%. *Methylobacterium* is gram negative bacteria and insensitive to penicillin.

There were significant differences in the bacterial genus diversity between the four samples with different concentration penicillin-G added. *Methylobacterium*, *unknown* bacteria, *Staphylococcus* and *Corynebacterium* were the former four kinds of bacteria that account for more abuntant in the water samples with penicillin G concentration of 10 ng/L. The abundance of *Staphylococcus* is about 20%, while the abundance were very low or can't be detected in other water samples. *Staphylococcus* is the most common suppurative cocci, and it is an important source of cross-infection in hospitals. *Corynebacterium*, *Dolosigranufum* and *Moraxela* were not found in other water samples.

The more abundant bacteria in the water samples with a penicillin-G concentration of 20 ng/L were *Methylobacterium* and *unknown* bacteria. The abundance of *Methylobacterium* is 64%, the highest in all

water sample. *Methylobacterium* and *unknown* bacteria were the most abundant bacteria in the water samples with a penicillin-G concentration of 50 ng/L, and the abundance of *Methylobacterium* is about 50%. The more abundant bacteria in the water samples with Penicillin-G concentration of 100 ng / L were *unknown*, *Methylobacterium* and *Rhodobacter*. *Rhodobacter* is a kind of gram negative bacteria and hasn't been detected in other water samples.

The diversity index of each sample was calculated. The results showed that the *Shannon* index of the water samples without penicillin-G was 2.823638, while the value decreased significantly after the addition of penicillin-G; *Shannon* index of water samples with penicillin-G added increased at first and then decreased slightly. *Shannon* index were the lowest at penicillin-G concentration of 20 ng/L, which is 0.961815, and increased with the concentration of penicillin-G. *Shannon* index is 1.689386 of water sample with penicillin-G concentration of 100 ng/L. This indicates that penicillin-G inhibits bacterial community and reduces the bacterial community diversity in drinking water.

4. Conclusions

1.   The microbial community structure had considerable changes after adding different concentrations of penicillin-G in drinking water. The proportion of *unknown* bacteria increased significantly, and the proportion of most of the bacteria decreased or even undetectable. The bacterial diversity in water samples with different concentration penicillin-G varied greatly, and each had its own specific bacteria.

2.   The diversity index of each sample was calculated. The results showed that the total number and abundance of bacterial community species in drinking water samples decreased significantly after the addition of penicillin-G. However, the number and abundance of microbial community structure did not change with the concentration of penicillin-G. Penicillin-G showed inhibitory effect on bacterial community, which reduced bacterial community diversity in drinking water.

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