Effect of disopyramide on bacterial diversity in drinking water

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Abstract: Disopyramide was detected in drinking water by LC-MS/MS and the microbial diversity was investigated by PCR and high-throughput sequencing. The results showed that bacteria community structure in drinking water changed a lot when added different concentrations of disopyramide. The results of Shannon index showed that the total number and abundance of bacterial community species in drinking water samples decreased significantly after the addition of disopyramide. However, the number and abundance of community structure did not change with the concentration of disopyramide. Disopyramide inhibits the activity of bacterial community in drinking water and also can reduce the bacterial community diversity in drinking water.

1. Introduction
Pharmaceuticals and personal care products (PPCPs) are a new type of environmental pollutant, and they are closely linked to human life. PPCPs include prescription and over-the-counter medicines (such as antibiotics, anti-inflammatories, antipyretics, analgesics, etc.), fragrances, cosmetics, sunscreens, diagnostics and nutraceuticals for human health or cosmetic care, similar products for livestock growth or health and so on [Qiao et al., 2009; Liu et al., 2009]. PPCPs with strong biological activity, glare and polarity, can enter the environment in various forms and ways. In recent years, PPCPs have been continuously detected in sewage treatment plants, surface water, groundwater and even drinking water [Mohapatra et al., 2016; Ternes et al., 2004]. Although the concentration of PPCPs in water bodies are low and their half-life is not very long, PPCPs can cause "fake persistence" and threaten human health [Vieno et al., 2005] due to the large and frequent use of individuals and livestock husbandry.

At present, there are no treatment process especially for PPCPs in most of the traditional drinking water treatment plants, and PPCPs can be removed completely by existing treatment processes. The frequently detection of PPCPs in drinking water indicated that the safety of potable water will be threatened [Pinkston et al., 2004].

Disopyramide as an anti-arrhythmic drug was used as a medication for the treatment of ventricular tachycardia either orally or injections since the 1970s. Yu detected PPCPs in water source of a city, the result showed that disopyramide is a typical substance in the detection frequency and concentrations [Yu et al., 2010]. The result of our research about PPCPs detection of the drinking water distribution network also showed that disopyramide was a content of high detection frequency and high concentration [Zhang, 2014]. Therefore, disopyramide was chosen and the interaction between disopyramide and bacteria diversity in drinking water was studied.

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 disopyramide (purchased from TRC Corporation, 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 disopyramide 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 mg/L |

2.2 Determination of disopyramide
The samples were enriched by solid-phase extraction (SPE), which used Oasis HLB cartridges (6 mL/500 mg, Waters, USA). The eluate was collected in a test tube and was evaporated using nitrogen sparging. Finally, 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 disopyramide in water samples, a tube wall adsorption experiment was performed, and the results showed that the effects of wall adsorption on disopyramide 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 and stored at −20°C. The bacterial 16S rRNA (V3+V4) genes were amplified, and bacterial diversity in the samples was detected by Illumina HiSeq 2000 and analyzed by Mothur software.

3. Results and discussion

3.1 Concentration of disopyramide
The concentrations of disopyramide in blank control and water samples with disopyramide added were detected by LC-MS/MS after 20 days, and the results were shown in table 2. There was no disopyramide detected in the blank control.
Table 2: Concentrations of disopyramide in drinking water samples (ng/L).

| Added disopyramide concentration | 10  | 20  | 50  | 100 |
|----------------------------------|-----|-----|-----|-----|
| Blank control                    | 9.1 | 18.3| 46.4| 96.1|
| Water samples with disopyramide added | 8.0 | 16.9| 43.7| 95.0|
| Value differences                | 1.1 | 1.4 | 2.7 | 1.1 |

As can be seen from Table 2, the concentration of disopyramide in non-sterilized drinking water samples was slightly 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 disopyramide.

3.2 Bacteria diversity

3.2.1 Bacteria diversity analysis of water sample without disopyramide added  Total DNA was extracted from the drinking water samples without added disopyramide, and the bacterial diversity was analyzed. GWS2 in Figure 1 is the bacteria diversity at genus level of water samples without disopyramide added. From the figure we can see that the abundance of unknown bacteria is about 30%, Sphingopyxis accounted for about 12%, Sphingomonas accounted for about 10%, Rhodobacter accounted for about 8%, Hyphomicrobium accounted for about 7%, Porphyrobacter accounted for about 7%, Uncultured Brucells and Lactococcus accounted for about 4%. Blastomonas, Bradyrhizobium, Staphylococcus, Pseudomonas, uncultured bacterium and others bacteria also accounted for a relatively small proportion.

3.2.2 Bacterial diversity analysis of water sample  After different concentrations of disopyramide 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). DSP1 to DSP4 represents disopyramide at concentration of 10ng/L, 20ng/L, 50ng/L and 100ng/L, respectively. From Figure 1 we can see bacteria abundance of each water samples.
After different concentrations of disopyramide were added in drinking water samples, bacteria community structure changed a lot. As we can see from Figure 1, the proportion of unknown bacteria increased 33%-45% compared with that of blank control (30%), and the highest concentration were in water sample with 20ng/L disopyramide added. The proportion of most genus dropped significantly and some genus even can’t be detected. The abundance of Rhodobacter, Hyphomicrobium, Porphyrobacter, uncultured Brucells and Phenyllobacterium decreased evidently. Lactococcus and Sphingopyxis presented in all four concentrations of disopyramide added samples, but the abundance were different. The abundance of Sphingopyxis increased significantly in water sample of 50ng/L disopyramide added. Methylobacterium didn’t exist in water sample without disopyramide added, but it appeared in water sample of 50ng/L and 100ng/L disopyramide added.

As a kind of new microbial resource, Sphingomonas can be used for the biodegradation of aromatic compounds due to its extensive metabolic capacity for such material. Some strains of the genus can synthesize valuable extracellular bio-polymer. With its high metabolic capacity and multi-physiological characteristics, Sphingomonas has great potential in environmental protection and industrial production. Methylobacterium is a Gram-negative bacteria and can use glucose or other complex nutrients as carbon and energy sources.

3.2.3 Bacterial diversity index The diversity index of each sample was calculated. The results showed that the total number and abundance of bacterial community in drinking water samples decreased significantly after the addition of disopyramide. However, the number and abundance of community structure did not change with the concentration of disopyramide. The shannon diversity index of the blank control water sample was 2.823638, while the diversity index was significantly decreased after the addition of disopyramide. The shannon index was the lowest at the concentration of water sample...
with 20ng/L of disopyramide added, and the value is 0.294381. The shannon index increased slightly, and reached to 0.378610 at concentration of water sample with 100ng/L of disopyramide added. This indicates that disopyramide inhibits the activity of bacterial community in drinking water and reduces the bacterial community diversity.

4. Conclusions
1. The microbial community structure had considerable changes after the addition of different concentrations of disopyramide in drinking water. The proportion of unknown bacteria increased significantly, and the proportion of most of the bacteria decreased or even undetectable. Lactococcus and Sphingopyxis presented in four concentrations of disopyramide water samples. Methylobacterium didn’t exist in water sample without disopyramide added, but it appeared in water sample of 50ng/L and 100ng/L disopyramide added.
2. The abundance of bacterial community species in drinking water samples decreased significantly after the addition of disopyramide. Disopyramide showed a general inhibitory effect on bacterial community, which reduced bacterial community diversity in drinking water.

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