

Efficient detection and assessment of human exposure to trace antibiotic residues in drinking water

Yujie Ben ^{a, b, c}, Min Hu ^{a, b}, Xingyue Zhang ^{a, b}, Shimin Wu ^d, Ming Hung Wong ^{a, b, e},
Mingyu Wang ^c, Charles B. Andrews ^{a, b}, Chunmiao Zheng ^{a, b, *}

^a Guangdong Provincial Key Laboratory of Soil and Groundwater Pollution Control, School of Environmental Science and Engineering, Southern University of Science and Technology, Shenzhen, 518055, China

^b State Environmental Protection Key Laboratory of Integrated Surface Water-Groundwater Pollution Control, School of Environmental Science and Engineering, Southern University of Science and Technology, Shenzhen, 518055, China

^c College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, 100190, China

^d IER Environmental Protection Engineering Technology Co., Ltd., Shenzhen, 518071, China

^e Consortium on Health, Environment, Education and Research (CHEER), Department of Science and Environmental Studies, The Education University of Hong Kong, Tai Po, Hong Kong, China

ARTICLE INFO

Article history:

Received 1 December 2019

Received in revised form

24 February 2020

Accepted 6 March 2020

Available online 11 March 2020

Keywords:

Antibiotic residues

Drinking water

Exposure assessment

Microbiological toxicity

Risk assessment

ABSTRACT

Human exposure to antibiotic residues in drinking water has not been well evaluated. This study is the first attempt to simultaneously and efficiently identify and quantify 92 antibiotic residues in filtered tap water (multistage filtration at the tap) ($n = 36$) collected from 10 areas of a large city in southern China, 10 Chinese brands of bottled/barreled water ($n = 30$) and six foreign brands of bottled water ($n = 18$) obtained from the Chinese market. The average and median concentrations of all the detected antibiotic compounds was 182 and 92 ng/L in filtered tap water, 180 and 105 ng/L in Chinese brands of bottled/barreled water, and 666 and 146 ng/L in foreign brands of bottled water, respectively. A total of 58 antibiotics were detected in the filtered tap water, and 45 and 36 antibiotics were detected in the Chinese and foreign brands of bottled water, respectively. More types of antibiotics were detected in Chinese brands of bottled water than in the other bottled waters. In addition, Chinese waters had high roxithromycin concentrations, while the foreign brands of bottled water had high concentrations of dicloxacillin. The average and median values of the estimated overall daily intake of all the detected antibiotics were 4.3 and 2.3 ng/kg/day when only filtered tap water was drunk, 4.0 and 2.5 ng/kg/day when Chinese brands of bottled water was drunk, and 16.0 and 4.9 ng/kg/day when foreign brands of bottled water was drunk. Further study is needed to develop a more comprehensive estimation of human exposure to antibiotic residues in the environment and a more in-depth understanding of the potential hazard of ingested antibiotic residues to the human microbiome.

© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Antibiotics are emerging contaminants of concern in the environment due to their potential adverse effects on nontarget organisms and increased bacterial resistance (National Academies of Sciences and Medicine, 2018; Qiao et al., 2017). The widespread use of human and veterinary antibiotics has resulted in a continuous release of antibiotics into the environment (Kumar et al., 2012; Zhang et al., 2015). A variety of antibiotic residues have been detected in

surface water and wastewater at concentrations ranging from 0.01 to 1.0 µg/L (Le Page et al., 2017). There is a high probability that antibiotics have already contaminated and will very likely remain in drinking water sources due to the lack of antibiotic removal capabilities of the current municipal drinking water and wastewater treatment infrastructure (Xu et al., 2007). The antibiotic residues consumed daily in drinking water interact with the human gastrointestinal microbiome. Long-term exposure to antibiotics may induce changes in the intestinal microflora structure and increase the emergence of antibiotic-resistant harmful bacteria or opportunistic pathogens inhabiting humans (Blaser, 2016; Damman et al., 2012; Pérez-Cobas et al., 2012).

Exposure to residual antibiotics in drinking water may cause adverse health risks on humans. Unlike in many nations (e.g., Japan

* Corresponding author. School of Environmental Science and Engineering, Southern University of Science and Technology, Shenzhen, 518055, China.

E-mail address: zhengcm@sustech.edu.cn (C. Zheng).

and the USA), China's tap water is not recommended for drinking due to chlorine disinfection. In recent decades, Chinese people have typically used boiled tap water for drinking. In recent years, an increasing number of people have gradually preferred to drink: 1) filtered tap water (i.e., tap water with on-site purification by multistage filters installed in drinking fountains), 2) bottled/barreled water produced by Chinese bottled water companies, and 3) bottled water from foreign countries. Consuming different types of drinking water may result in different levels of antibiotic residue exposure. However, only a few studies have focused on human exposure to antibiotics via drinking water (Benotti et al., 2009; Boleda et al., 2014; Cai et al., 2015; Ji et al., 2010; Li et al., 2017; Wang et al., 2016; Yiruhan et al., 2010; Simazaki et al., 2015; Leung et al., 2013; Watkinson et al., 2009; Zuccato et al., 2000) (Table 1), and these studies have had two limitations. First, only 2 to 28 kinds of antibiotics have been investigated in drinking water, and only 20 were detected (Table 1). Given that 120 different antibiotics are used in human health care and livestock husbandry (Wang et al., 2015), it is highly likely that antibiotic residues in drinking water are greater than the range of investigated antibiotics. Second, most previous studies assessed only tap water and ignored possible

human exposure to antibiotic residues in other types of drinking water. These two limitations may have resulted in a biased underestimate of human exposure and health risk of antibiotic residues in drinking water (Benotti et al., 2009; Boleda et al., 2014; Simazaki et al., 2015; Yiruhan et al., 2010; Zuccato et al., 2000).

The quality of drinking water is essential to human health and social development. Thus, it is paramount to gain a comprehensive understanding of the potential human health risks due to antibiotic residue exposure from drinking water. To that end, this study monitored a wider range of antibiotic residues (over 100 kinds) in filtered tap water, and bottled and barreled water than other studies, and comprehensively assessed human exposure to residual antibiotics in different types of drinking water.

2. Methods

2.1. Collection of drinking water samples

Drinking water samples were collected from a southern coastal city of China. The city has a large population density (approximately 2000 square kilometers and 13 million permanent

Table 1
Antibiotic concentrations in the drinking water in previous studies.

Sampling Region	Sampling Year	Sample Type	Analytes	Concentration (ng/L): Mean (range, detection rate)	Reference
Shanghai, China	2015	Tap water (n = 46) Bottled water (n = 45) Barreled water (n = 8)	21 ^a	Florfenicol: 11 ng/L (0.82–24, 100%), thiamphenicol: 7.6 ng/L (0.84–23, 100%) Florfenicol: N.A. (0.6–1, 6.67%) N.D.	Wang et al. (2016)
Guangzhou, China	2008	Tap water from 10 districts (3–5 replicates)	4 ^b	Norfloxacin: 15 ng/L (N.D.–82.7, 90%), ciprofloxacin: 270 ng/L (6–679.7, 100%), lomefloxacin: 59.3 ng/L (N.D.–179, 70%), enrofloxacin: 2 ng/L (N.D.–8.3, 50%)	Yiruhan et al. (2010)
Marco, China		Tap water from 12 districts (3–5 replicates)		Norfloxacin: 9 ng/L (7.1–17.1, 100%), ciprofloxacin: 4 ng/L (2–8.2, 100%), lomefloxacin: 24 ng/L (8.9–37.1, 100%), enrofloxacin: 4 ng/L (2.8–5.2, 100%)	
Japan	2006–2010	Finished drinking water (N.A.)	8 ^c	N.D.	Simazaki et al. (2015)
Spain	2008–2009	Finished drinking water (n = 50)	15 ^d	Azithromycin: 0.9 (N.D.–9.5, 10%), clarithromycin: 0.2 (N.D.–2.2, 10%), erythromycin: 0.2 (N.D.–1.5, 16%), sulfamethoxazole: N.D. (N.D.–1.4, 2%)	Boleda et al. (2014)
China	2010–2011	Tap water (n = 113)	9 ^e	Clarithromycin: 6.7 ^f (N.D.–11.9, 7.1%), roxithromycin: 2.8 ^f (N.D.–15.1, 7.1%), azithromycin: 7 ^f (N.D.–11.7, 7.1%), sulfathiazole: N.D. (N.D.–27.4, 0.88%), sulfamethazine: 9.4 ^f (N.D.–89.6, 5.3%), sulfamethoxazole: 8 ^f (N.D.–21.2, 8.9%), thiamphenicol: 17.8 ^f (N.D.–104.3, 11.5%), trimethoprim: 10.2 ^f (N.D.–14.2, 1.8%), tylosin: 6.4 ^f (N.D.–7.0, 3.5%)	Leung et al. (2013)
USA	2006–2007	Finished drinking water (n = 18) Tap water (n = 15)	2	Sulfamethoxazole: 0.39 ^f (N.D.–3.0, 22%), trimethoprim: N.D. Sulfamethoxazole: N.D. (N.D.–0.32, 6.7%), trimethoprim: N.D.	Benotti et al. (2009)
Beijing, China	2013	Tap water (N.A.)	2	Sulfamethoxazole: N.A. (N.D.–1.81, 32.5%), erythromycin: N.D.	Cai et al. (2015)
Australia	2005–2006	Drinking water (n = 20)	28 ^f	N.D.	Watkinson et al. (2009)
Korea	2007	Drinking water (N.A.)	6 ^g	N.D.	Ji et al. (2010)
Tai Po, Hong Kong	2016	Tap water (N.A.)	13 ^h	Oxytetracycline: 600 ^m (N.A., N.A.)	Li et al. (2017)
Italy	N.A.	Drinking water (N.A.)	7 ⁱ	Tylosin: N.A. (N.D.–1.7, N.A.)	Zuccato et al. (2000)
Italy	2004	Bottled water (n = 8)	11 ^j	Sulfamethizole: N.A. (N.D.–9, 12.5%), sulfadimethoxine: N.A. (N.D.–11, 12.5%), sulfamethoxazole: N.A. (N.D.–80, 25%)	Perret et al. (2006)
Spain	2005–2006	Bottled water (N.A.)	10 ^k	Sulfadimethoxine: 0.164 (N.A., N.A.), N-acetylsulfamethazine: 1.1 (N.A., N.A.)	Díaz-Cruz et al. (2008)

^a 5 macrolides, 2 β -lactams, 3 tetracyclines, 4 fluoroquinolones, 4 sulfonamides, and 3 chloramphenicols.

^b 4 quinolones.

^c 2 macrolides, 3 tetracyclines, and 3 sulfonamides.

^d 3 tetracyclines, 6 macrolides, 1 β -lactams, 4 sulfonamides, and 1 fluoroquinolones.

^e 4 macrolides, and 5 sulfonamides.

^f 7 macrolides, 6 β -lactams, 4 tetracyclines, 4 fluoroquinolones, 4 sulfonamides and 3 polypeptides and other antibiotics.

^g 1 macrolides, 1 fluoroquinolones, and 4 sulfonamides.

^h 2 macrolides, 4 fluoroquinolones, 3 sulfonamides, 1 β -lactams, and 3 tetracyclines.

ⁱ 6 macrolides, and 1 β -lactams.

^j 11 sulfonamides.

^k 9 sulfonamides and 1 metabolite.

^l Concentration (ng/L): median.

^m For tap water, in this study, only one TC (OTC, 0.6 ng/mL) was detected in terminal tap water in Hong Kong.

residents) and a well-developed economy. The tap water in the city is mainly derived from the East River (Dongjiang), one of the main streams of the Pearl River Delta, and is not considered to be directly drinkable without treatment. Bottled water and barreled water drinking fountains are of widespread availability in office buildings and residences, and are the most common sources of drinking water. In addition, there are a few high-end residential areas, public institutions and facilities and commercial office buildings that have installed centralized treatment systems for further filtration of tap water. Meanwhile, many families have purchased household tap water filtration/purification systems. Typical commercial and residential tap water treatment systems consist of four filtrations steps: 1) polypropylene cotton, 2) activated carbon, 3) core purification membrane, and 4) a final activated carbon step to remove odor. There are mainly three types of core filtration membranes in the Chinese market: ultra-filtration membrane (UF), nano-filtration membrane (NF), and reverse osmosis membrane (RO). The filtration accuracy of UF, NF, and RO is 0.01, 0.001, and 0.0001 μm , respectively. At present, RO is the most advanced and popular tap water treatment systems in the Chinese market. Generally, high-end residential areas, public institutions and facilities and commercial office buildings are equipped with RO drinking fountains. Residential tap water treatment systems are widely available for purchase in the city. Many households use RO drinking systems while others choose the other two types of core purification membranes due to lower price. The life span of the RO membrane depends on the quality of the tap water. The manufacturers recommend replacing the membrane once a year.

Thus, three types of drinking water were collected, including filtered tap water, Chinese brands of bottled and barreled water, and foreign brands of bottled water. In addition, unfiltered tap water samples were also collected.

2.1.1. Filtered tap water

There are approximately 44 water reservoirs supplying tap water in the city. The water sources for these reservoirs are from the upper reach of the East River (Dongjiang) and local precipitation. The entire city was divided into 10 areas for collecting direct drinking water samples. Samples, 3 or 6 samples individually sized with the volume of 1 L, were collected in each area in January 2019 and April 2019. The sampling points selected were district libraries or office buildings that are equipped with drinking fountains to supply filtered tap water. A total of 36 filtered tap water samples were collected. Unfiltered tap water samples ($n = 30$) at the same sampling points as the filtered tap water samples were also collected as a reference.

2.1.2. Bottled or barreled water

A total of 42 bottled water samples from 14 brands were purchased from convenience stores or online shopping malls, with 3 individual bottled water samples with the volume of 1 L collected for each brand. The 14 brands of bottled water include the eight most common brands in China and six foreign brands (two from France, one from Japan, one from Italy, one from Germany, and one from the USA). Since most of the barreled water was of the same brand as the bottled water, only 2 brands of barreled water were collected with 3 1-L individual samples collected for each brand. All water samples were kept at 4 °C and were analyzed within 3–7 days.

2.2. Selection of target analytes

Due to the lack of official data on the production or use of antibiotics in China, a total of 106 antibiotics were included as target chemicals in the present study based on reported antibiotics in the

environment and food (Ben et al., 2019). They covered the most commonly used antibiotics used for human care and animal husbandry, including 25 sulfonamides, 5 acetylated sulfonamides, 20 quinolones, 6 tetracyclines and 9 of their degradation products, 16 macrolides, 14 β -lactams, 3 chloramphenicols, 3 polypeptides and 5 other antibiotics. Nine stable isotope-labeled internal standards were used for analyzing the 106 antibiotics, including $^{13}\text{C}_6$ -sulfa-dimidine, d_4 -acetylsulfamethoxazole, ^{13}C , d_3 -erythromycin, d_5 -norfloxacin, d_4 -fluconazole, d_5 -metoprolol, d_2 -carbamazepine, $^{13}\text{C}_3$ -trimethoprim, and d_3 -triclosan. For detailed information and standard sources of the chemicals, see **Supplementary Materials: Chemicals and Regents**.

2.3. Sample preparation

The sample preparation followed an established method for water samples (Wang et al., 2016; Yiruhan et al., 2010). Briefly, each 1-L water sample was filtered through 7 μm GF (glass microfibre filter)/F (Whatman, Buckinghamshire, UK). Then, 0.5 g Na_2EDTA and isotope-labeled mixed internal standards were added to the filtrate. The targeted antibiotic compounds in the drinking water sample were enriched using a hydrophilic lipophilic balanced (HLB) solid-phase extraction (SPE) cartridge (Oasis, 500 mg/6 mL, Waters, Milford, MA, USA) following the procedure described below. The HLB cartridges were preconditioned with 10 mL methanol and 10 mL Milli-Q water in sequence. After acidification to $\text{pH} = 3 \pm 0.5$ with 1 mol/L hydrochloric acid, the water samples were loaded on the cartridge at a flow rate of 5–10 mL/min. Then, the cartridges were rinsed with 10 mL Milli-Q water and vacuum-dried for approximately 20 min. The analytes were eluted from the HLB cartridge using 10 mL methanol. The elution was evaporated to dryness under a gentle stream of nitrogen using a Turbopap (Biotage, Cardiff, UK) at 40 °C and reconstituted with 0.5 mL methanol for instrumental analysis.

2.4. Instrumental analyses

An Agilent 1290 Infinity Ultra-High-Performance Liquid Chromatography equipped with an Agilent 6470 Triple Quadrupole Mass Spectrometer (UHPLC-MS/MS) was used for targeted identification and quantification of the 106 antibiotics in drinking water. The MS conditions were optimized for detecting the 106 antibiotics, and two precursor-fragment ion pairs with the highest abundances were generally selected for mass spectrometric analysis. The ion pairs, fragmentation voltages and collision energies for the detected antibiotics and the ^{13}C -or ^2H -labeled internal standard were determined (see Supplementary Materials, Table S1). Based on the different physical-chemical properties of the targeted analytes, the 106 antibiotics were divided into two groups: one comprised 102 antibiotics, and another comprised the remaining 4 antibiotics, including three chloramphenicols and one sulfanilamide.

The 102 antibiotics in the first group were analyzed on a Waters BEH C8 column (1.7 $\mu\text{m} \times 2.1 \times 100$ mm, Waters, Milford, MA, USA) with a vanguard column. To obtain a better separation of target analytes, different mobile phases were applied in this study. Although sharp peaks and improved sensitivity of analytes can be obtained when using the mobile phase (A: 0.1% formic acid in water; B: acetonitrile), four isomers, including epioxytetracycline, oxytetracycline, sulfameter and sulfamethoxypyridazine, could not be separated due to poor chromatographic separation. By switching the mobile phase B from acetonitrile to methanol, these isomers could be well separated in the chromatogram (see Supplementary Materials, Fig. S1). Mobile phases A and B were 0.1% formic acid in water and methanol, respectively. The flow rate was 0.40 mL/min, and the column was kept at room temperature (25 °C). The gradient

conditions were as follows: 0 min, 10% B; 3.5 min, 40% B; 4.0 min, 48% B; 4.5 min, 30% B; 6.0 min, 100% B; 8.0 min, 100% B; 10.01 min 10% B and re-equilibration to 14 min. The antibiotics were identified and quantified by dynamic multi-reaction monitoring (MRM) in ESI⁺ mode. The injection volume was 2 μ L. For the mass spectrometer settings, the gas and sheath gas temperatures were 325 and 350 $^{\circ}$ C, and the flow rates were 10 and 11 L/min, respectively. The nebulizer was set at 20 psi. The capillary and nozzle voltages were 4000 and 2000 V, respectively. In this way, ofloxacin and levofloxacin, a pair of chiral compounds, were very difficult to separate on an achiral chromatographic column using methanol or acetonitrile as the mobile phase. The total levels of these two compounds are presented as ofloxacin/levofloxacin in the present study.

The remaining four antibiotics in the second group were analyzed by MRM in ESI⁻ mode. A Waters CORTECS T3 column (1.6 μ m $\times$ 2.1 $\times$ 100 mm, Waters, Milford, MA, USA) with a vanguard column was used for chromatographic separation. Mobile phases A and B were ultrapure water and methanol, respectively. The flow rate was set at 0.40 mL/min, and the column was operated at room temperature (25 $^{\circ}$ C). The gradient conditions were as follows: 0 min, 10% B; 1 min, 70% B; 3 min, 90% B; 4 min 10% B, and re-equilibration to 6 min. The injection volume was 5 μ L. The MS conditions were set as the same as those for the analysis of the antibiotics in the former group.

2.5. Limits of detection (LODs)

The instrument detection limits (IDLs) were defined as follows:

$$IDL = t \times RSD \times \frac{\text{amount injected}}{100} \quad (1)$$

where t is the Student's " t " value given for the specified confidence level (99%) and degree of freedom ($n-1$) and RSD is the relative standard deviation of peak area at the amount injected from n replicate injections, generally $n \geq 6$.

One negative control (water blank spiked with the internal standards and run through the identical analysis procedures as those for the actual samples) was determined along with every 6 samples, and a total of 15 negative control samples were analyzed. Background noise interfered with the signals of bacitracin A, doxycycline, fleroxacin, iso-chlortetracycline, methacycline, minocycline, monensin, ofloxacin/levofloxacin, pipemidic acid, piperacillin, roxithromycin, sulfadiazine, sulfamethoxazole, tetracycline, trimethoprim, and sulfantran. The limits of detection (LODs) for these compounds in the blank samples were calculated as the mean plus three standard deviations of the concentrations and ranged from 0.031 to 24 ng/L. For those compounds that were not detected in water blanks, the LODs were set equal to the IDLs. As a result, the LODs ranged from 0.030 to 0.28 ng/L for sulfonamides, 0.040–0.50 ng/L for quinolones, 0.022–3.5 ng/L for tetracyclines, 0.0090–0.41 ng/L for macrolides, 0.022–24 ng/L for β -lactams, 0.023–0.073 ng/L for chloramphenicols, and 0.012–0.063 ng/L for the other antibiotics in drinking water (see Supplementary Materials, Table S2).

2.6. Data analyses

Identification and quantitation of the analytes were performed using Agilent's Mass Hunter Quantitative Analysis Software (version B.08.00). To avoid overestimation of human exposure to antibiotics via drinking water, we assumed the amounts for undetected antibiotics below the LODs equal to 0 if the detection frequency of antibiotics were <50%, while the amounts were

assumed to be LOD/2 if the detection frequency of antibiotics were >50% in the present study. To characterize population variability of antibiotic daily intakes via drinking water, bootstrap re-sampling-based Monte Carlo simulation was performed with 5000 iterations by Matlab (version 2015a). The cumulative distribution function of the 5000 iterations showed that more than 90% of our generated data can well fit the characteristics of statistical distributions of observations. The statistical descriptors of the intakes of the detected compounds in drinking water including the mean and median were estimated on the basis of the 5000 iterations.

3. Results and discussions

3.1. Method development for the quantitation of residual antibiotic in drinking water

This study is the first attempt to simultaneously determine approximately 100 antibiotics in drinking water. Based on the different physical chemical properties of the targeted analytes, the 106 antibiotics were divided into two groups: 102 antibiotics were efficiently separated and detected within 10 min using water (0.1% FA)/methanol as mobile phases in dynamic MRM ESI⁺ mode with a Waters BEH C8 column, and the remaining 4 analytes were identified and quantified within 5 min by MRM in ESI⁻ mode with a Waters CORTECS T3 column. The optimized chromatograms of the 106 antibiotics and sub-chromatograms for 7 classes of antibiotics are shown in Fig. 1, showing clear quantifiable peaks for all the analytes. The recovery validation was carried out in 3–6 replicates of 1 L filtered tap water spiked with three levels (0.5, 5 and 50 ng/L) of antibiotic mixture standards. In contrast to 14 analytes with recovery rates always lower than 40%, the average recovery of 92 analytes was (mean $\pm$ standard deviation) $88.0 \pm 19.1\%$, $80.0 \pm 23.9\%$, $88.2 \pm 37.9\%$ at the spiked levels of 50, 5, and 0.5 ng/L, respectively, while the 0.5 ng/L spiking was close to the LODs of some analytes. The 14 analytes with low recoveries were bacitracin A, colistin A, colistin B, α -apo-oxytetracycline, β -apo-oxytetracycline, sulfasalazine, spiramycin, sulfaguanidine, sulfanilamide, narasin, salinomycin, tilmicosin, 4-epianhydrochlortetracycline, and 4-epianhydrotetracycline (the chromatograms of spiked narasin and salinomycin showed 2 peaks after pretreatment). Therefore, 92 analytes were identified and quantified in the following analysis of antibiotics in the samples of filtered tap water, Chinese bottled and barreled waters, and foreign brands of bottled waters.

It is noteworthy that in unfiltered tap water, there were only 30 analytes with a recovery rate higher than 40%, with an average recovery rate for the 30 analytes of $81 \pm 26.0\%$ and $80 \pm 23\%$ at the spiked levels of 50 and 5 ng/L, respectively. The identification and quantitation of the remaining 76 analytes were strongly inhibited by impurities in tap water (see Supplementary Materials: Fig. S2), with average matrix spike recovery rates of $9.8 \pm 1.3\%$ and $5.0 \pm 0.9\%$ at spiked levels of 50 and 5 ng/L, respectively. The low recovery rates for the 76 analytes is likely caused by interference from disinfection byproducts in tap water and secondary pollution generated from pipeline aging. These results were consistent with a previous research finding that the SPE efficiency, quantification and quantitation of twelve sulfonamides were strongly influenced by the matrix effect of tap water (Borecka et al., 2014). Analyte recoveries for the filtered tap water samples exhibited significantly higher recoveries (as described above) of analytes in matrix-spiked experiments than unfiltered tap water, suggesting that the filtration and purification technology used for the filtered tap water could be good for pretreatment to reduce matrix effects when antibiotics are analyzed in tap water.

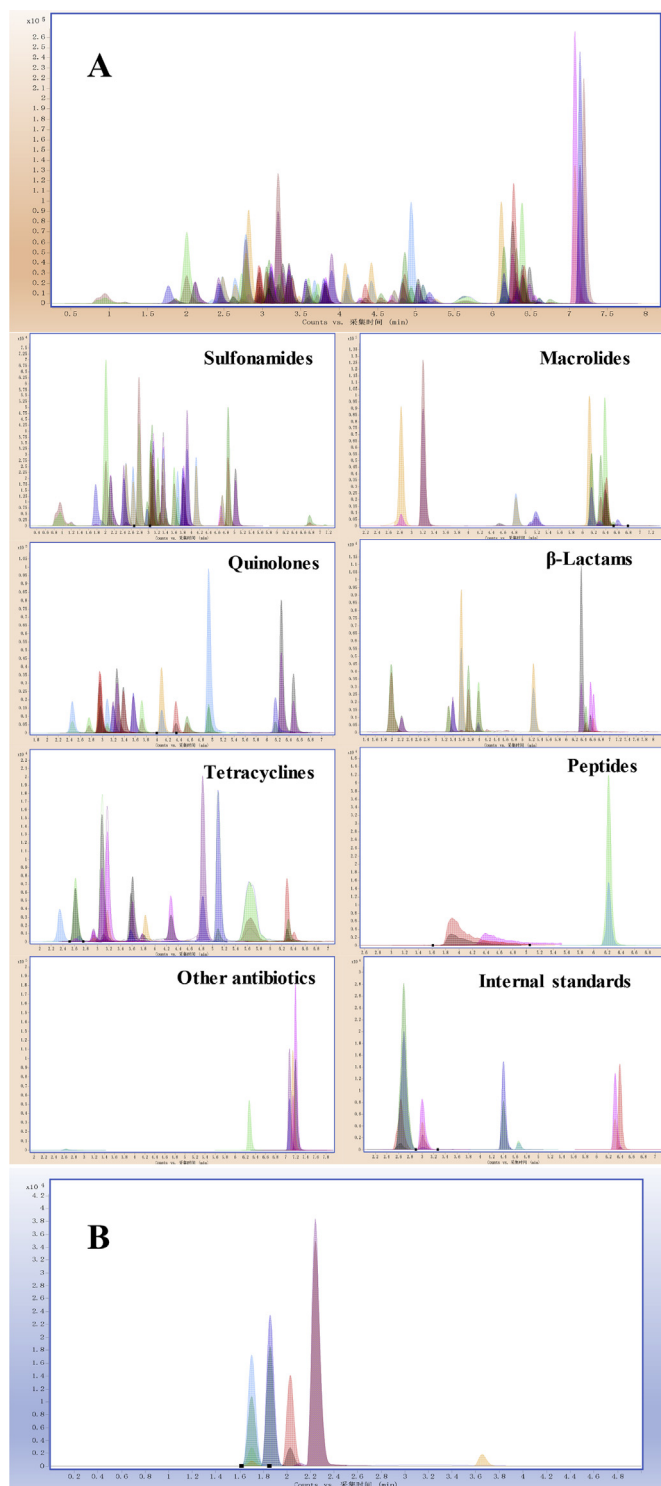


Fig. 1. The complete chromatogram of the 106 antibiotics combined with the extracted subgraphs of different classes of antibiotics: (A) 102 antibiotics and (B) three chloramphenicols and sulfanitran.

3.2. Occurrence of antibiotics in drinking water

3.2.1. Filtered tap water

The filtered tap water samples were collected from 10 areas of the city, whose water supply comes from different reservoirs. Five or more compounds were detected in all the filtered tap water

samples. The highest number of detected analytes was 41 at one sampling site whose water supply was from a reservoir surrounded by restaurants, indicating that the water source in the reservoir may be polluted by the dining industry sewage. The concentrations of the detected compounds ranged from 0.021 ng/L for jossamycin to 1,133 ng/L for dicloxacillin. Among the 92 analytes, 34 were not detected, and 37 were detected in fewer than 30% of samples (range: 3%–28%). Twenty-one were detected in more than 30% of filtered tap water samples (with the highest detection frequency being 100% for trimethoprim) (Fig. 2). Nineteen parent compounds (four tetracyclines, three macrolides, six quinolones, four sulfonamides, one β -lactam, and monensin) and two degradation products (4-epitetracycline and N-acetylsulfamethoxazole) were detected in the 36 filtered tap water samples, with an average and median concentration ranging from 0.24 and N.D. ng/L for sulfamethoxazole to 101 and 25 ng/L for dicloxacillin (see Supplementary Materials: Table S2). The total concentration of the detected antibiotics in filtered tap water samples ranged from 6.0 ng/L to 1172 ng/L with an average and median concentration of 182 and 92 ng/L, and 80% of the filtered tap water samples had a total concentration of detected antibiotics of greater than 50 ng/L. In addition, the 30 antibiotic analytes that were not affected by the matrix effect of tap water ($n = 30$) were also quantitated in the unfiltered tap water samples collected at the same sampling sites as the filtered tap water samples (see Supplementary Materials: Fig. S3). Similar detection frequencies and concentrations of target analytes were observed in tap water and filtered tap water samples ($p > 0.05$). This may suggest that the multistage filter in regular drinking fountains involved in the present study does not remove antibiotics at the residual level of ng/L.

There are relatively limited studies regarding antibiotic occurrence in drinking water (Cai et al., 2015; Hanna et al., 2018; Leung et al., 2013; Li et al., 2017; Yiruhan et al., 2010), and the available data are reviewed in Table 1. Compared with the concentrations in these studies, the maximum concentrations of azithromycin (22 ng/L), clarithromycin (8.9 ng/L), enrofloxacin (13 ng/L), and trimethoprim (5.2 ng/L) in the collected filtered tap water samples were close to those in tap water from Shanghai, Changsha, Hangzhou, and Guangzhou, China (Leung et al., 2013). However, the maximum levels of lomefloxacin (1.2 ng/L) and sulfamethazine (3.2 ng/L) in our filtered tap water samples were significantly lower than the levels (179 ng/L and 77.2 ng/L, respectively) in tap water from Guangzhou, and the maximum levels of sulfamethoxazole (0.98 ng/L) and ciprofloxacin (5.7 ng/L) in our samples were lower than those in tap water from Guangzhou (21.2 ng/L and 679.7 ng/L, respectively) and Shandong (18.6 ng/L and 224.4 ng/L, respectively) (Yiruhan et al., 2010; Leung et al., 2013; Hanna et al., 2018). Furthermore, the maximum concentration of norfloxacin (297 ng/L) and roxithromycin (27 ng/L) in the collected filtered tap water samples was significantly higher than that (82.7 ng/L and 15.1 ng/L, respectively) in tap water from Guangzhou (Yiruhan et al., 2010; Leung et al., 2013). In contrast to the high concentrations of chloramphenicols detected in tap water from Shanghai (Wang et al., 2016), Hangzhou and Guangzhou (Leung et al., 2013), chloramphenicols were seldom detected in our filtered tap water samples. According to Announcement No. 193 listing the veterinary drugs and other compounds banned for livestock animals (Ministry of Agriculture of the People Republic of China, 2011), chloramphenicol has been listed as a banned veterinary drug since 2002, due to its inhibition of hematopoietic function of bone marrow. Clinical applications are also limited. However, due to its strong antibacterial effect and low price, it is likely that chloramphenicol is still in use illegally. The infrequent detection in the present study may indicate that the current use of chloramphenicol is gradually in decline due to strict control in recent years.

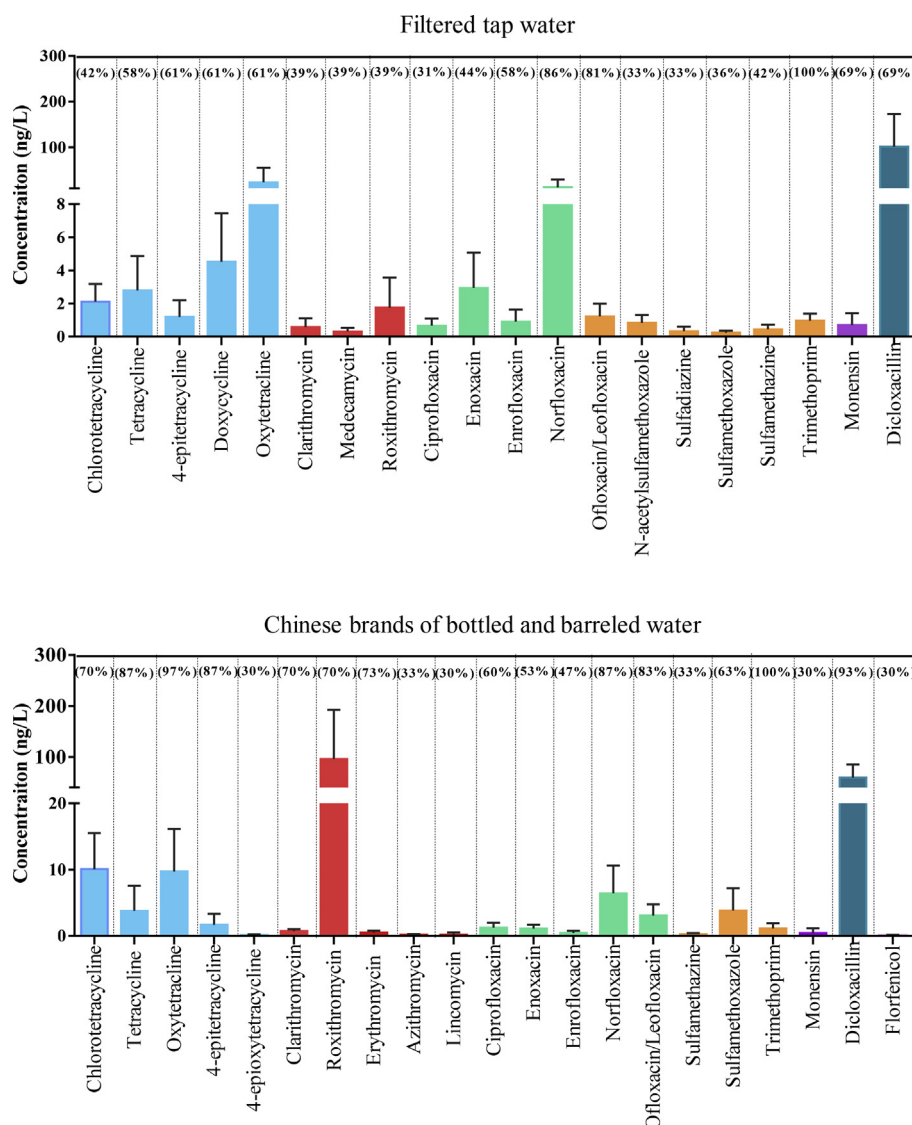


Fig. 2. The average concentrations with 95% confidence interval of the detected compounds with a detection frequency >30% in the 36 filtered tap water from 10 areas of the city and the 30 Chinese bottled/barreled water samples bought from the market. The percentages of samples with positive detections are shown in parentheses for each compound.

3.2.2. Chinese brands of bottled/barreled water

The 30 individual bottled/barreled water samples were from eight brands of bottled water and two brands of barreled water bottled by different companies in China. The water sources of the different brands of bottled water are distributed throughout the country. Four or more compounds were detected in the Chinese brand bottled and barreled water samples, with the highest number of detected compounds in one brand of bottle samples being 31. The concentration of the detected compounds ranged from 0.0094 ng/L for jossamycin to 1,376 ng/L for roxithromycin. Among the 92 analytes, 47 were not detected, and 23 were detected in fewer than 30% of the samples (range: 3%–27%). Twenty-two were detected in more than 30% of bottle water samples, with the highest detection frequency being 100% for trimethoprim. Similar to the results for filtered tap water; these twenty-two included 20 parent compounds (three tetracyclines, five macrolides, six quinolones, three sulfonamides, one β -lactam, one chloramphenicol, and monensin) and two degradation products (4-epitetracycline and 4-epioxytetracycline) (Fig. 2), with an average and median concentration ranging from 0.034 and N.D. ng/L for monensin to 96

and 5.5 ng/L for roxithromycin (see Supplementary Materials: Table S2), respectively. The total concentration of the detected antibiotics in bottled and barreled water samples ranged from 16 ng/L to 1,554 ng/L, with an average and median concentration of 180 and 105 ng/L, respectively. Additionally, 80% of the bottled and barreled water samples had a total concentration of detected antibiotics of greater than 50 ng/L. The Chinese brands of bottled water labeled as mineral water or purified water collected in the present study were derived from deep lakes, groundwater, or mountain springs in different regions of China. The production of these bottled water generally included: aeration-filtration - sterilization -filling. The types and concentrations of detected compounds in filtered tap water and bottled and barreled water were very similar (Fig. 2), which may indicate a background pollution of antibiotic residues in Chinese waters.

It is also noteworthy that eight of 10 degradation products were found in the collected water samples, while 50 of 82 parent compounds of antibiotics were detected in the water samples. This may indicate a wide occurrence of antibiotic degradation products in drinking water in China. Possible antibiotic degradation products in

the environment range far beyond the 10 quantitative analytes in the present study and even far beyond our current knowledge. Identification and quantification of unknown products from antibiotics transformation in the environment should receive more attention.

3.2.3. Foreign brands of bottled water

The foreign bottled water samples included one brand from the USA, one from Japan, one from Italy, one from Germany and two from France, each with 3 individual samples. Three or more compounds were detected in these foreign bottled water samples, with the highest number of detected antibiotic analytes in a single brand of bottled water samples being 23. The concentration of the detected compounds ranged from 0.013 ng/L for monensin to 3,462 ng/L for dicloxacillin. Among the 92 analytes, 56 were not detected, and 20 were detected in fewer than 30% of the samples (range: 6%–28%). Sixteen were detected in more than 30% of the samples, with the highest detection frequency being 100% for dicloxacillin and trimethoprim; these sixteen included 15 parent compounds (four tetracyclines, six quinolones, three sulfonamides, one β -lactam, and monensin) and one degradation product (4-epitetracycline) with average and median concentrations ranging from 0.068 and 0.060 ng/L for monensin to 631 and 113 ng/L for dicloxacillin (see Supplementary Materials: Table S2). The concentrations of sulfamethizole (N.D.), sulfadimethoxine (N.D.), and sulfamethoxazole (N.D.–0.38 ng/L) detected in the Italian brand of bottled water collected in the present study were lower than those (N.D.–9 ng/L for sulfamethizole, N.D.–11 ng/L for sulfadimethoxine, and N.D.–80 ng/L for sulfamethoxazole, respectively) reported in a previous study (Perret et al., 2006). The total concentration of the detected antibiotics in bottled water samples from these countries ranged from 69 ng/L to 3,486 ng/L with an average and median concentration of 666 and 146 ng/L; almost 90% of the total antibiotic concentration was contributed by dicloxacillin. If dicloxacillin was excluded, the average and median concentration of the overall detected antibiotics was 35 and 18 ng/L. This may be related to the use of dicloxacillin for the treatment of methicillin-susceptible *S. aureus* infections in human and small animal health care (Sandberg et al., 2010). Dicloxacillin is a member of penicillinase-resistant penicillins, and now is also used for therapy as a composite drug combined with amoxicillin, due to its stability against penicillinases and low toxicity. According to the World Health Organization report on surveillance of antibiotic consumption in 2016–2018 (WHO, 2018), dicloxacillin contributed about 7.3% of total defined daily doses (DDD) of antibiotics per 1000 inhabitants per day (oral intake) in the region of the Americas, and it was found that 65% of dicloxacillin by oral intake is excreted through urine and released into the environment (Hirsch et al., 1999).

3.2.4. Comparison between Chinese and foreign brands of bottled water

Compared with the antibiotics detected in these foreign brands of bottled water, the number of antibiotics detected in Chinese bottled water was significantly higher, indicating that the antibiotic pollution in Chinese drinking water was more complex. Macrolides were the most prevalent class of antibiotics detected in China (Fig. 3), and roxithromycin was detected in Chinese bottled water with a high frequency and concentration, while it was seldom detected in any foreign brands of bottled water. This may be due to the wide use of roxithromycin as a veterinary medicine in China. The concentrations of sulfonamides detected in Chinese bottled water were higher than that in the foreign brands of bottled water, while the concentration of quinolones was similar to that in the foreign brands of bottled water. The concentration of tetracyclines in water from China was lower than those in water from Germany

and France but higher than those in water from the USA and Japan. Chloramphenicols and other antibiotics were present in low concentrations and exhibited low detection frequencies in all bottled waters. It is noteworthy that the concentrations of β -lactams (dicloxacillin) detected in water from Germany and France were 10–30 times higher than those detected in water from China, the USA and Japan. However, the comparison of antibiotics in drinking water between China and other countries in the present study was limited by the sample size. Further nationwide and worldwide investigations of antibiotic residues in drinking water are warranted.

3.3. Assessment of human exposure to residual antibiotics in drinking water

3.3.1. Exposure assessment

Currently, Chinese people, especially urbanites, are increasingly demanding high-quality drinking water and thus prefer filtered tap water or bottled/barreled water, while some wealthy people even only drink bottled water produced by foreign nations. The daily exposure of people living in the city to residual antibiotics via drinking water was estimated for three scenarios. The first scenario was that people only drank filtered tap water (scenario 1), the second was that people only drank Chinese brands of bottled/barreled water (scenario 2), and the third one was that people only drank foreign brands of bottled water (scenario 3). Daily intake by drinking water (ng/kg/day) was calculated by the following equation:

$$DI_{\text{water}} = \frac{C_i D_{\text{water}}}{BW} \quad (2)$$

where C_i is the concentration of compound i in the drinking water sample and D_{water} and BW are the daily water intake and body weight, which were set to 1.7 L and 65 kg for the Chinese general population, respectively, according to the Major Science and Technology Program for Water Pollution Control and Treatment of China (2014–2017) (RCEESCAS, 2018; Zhang et al., 2019). There were still a variety of possibilities in the drinking water sources in each scenario. For example, in scenario 1, a resident in the city would be most likely to only drink from one or two areas (home and work), while the possibility of drinking water from all the 10 subareas is also possible. Similarly, many residents tended to drink the same brand of bottled water every day, while some people preferred to change the brands of bottle water. Thus, to characterize the variability of antibiotic daily intakes via drinking water, bootstrap re-sampling based on Monte Carlo simulation was performed with 5000 iterations. With Monte Carlo simulations based on an empirical cumulative probability function of the concentration data, the average and median estimated overall daily intakes of all the detectable antibiotics by drinking water were 4.3 and 2.3 ng/kg/day (0.14–31 ng/kg/day) for scenario 1, 4.0 and 2.5 ng/kg/day (0.40–41 ng/kg/day) for scenario 2, and 16 and 4.9 ng/kg/day (1.8–91 ng/kg/day) for scenario 3, respectively. Considering the possibilities that humans could be exposed to antibiotic residues in the environment by drinking water, inhalation, ingestion and dermal contact of particulate matter, and food consumption, the total daily intake of antibiotic residues for the people living in the city may be close to a level of $\mu\text{g/kg/day}$.

3.3.2. Risk assessment

Compared with effects of therapeutic clinical or self-medication as short-term and high-dose exposures, the effect of antibiotic residues from drinking water on humans is likely to be a long-term and low-dose exposure. The trace amounts of individual antibiotic residues in the environment may pose a low selection risk to the

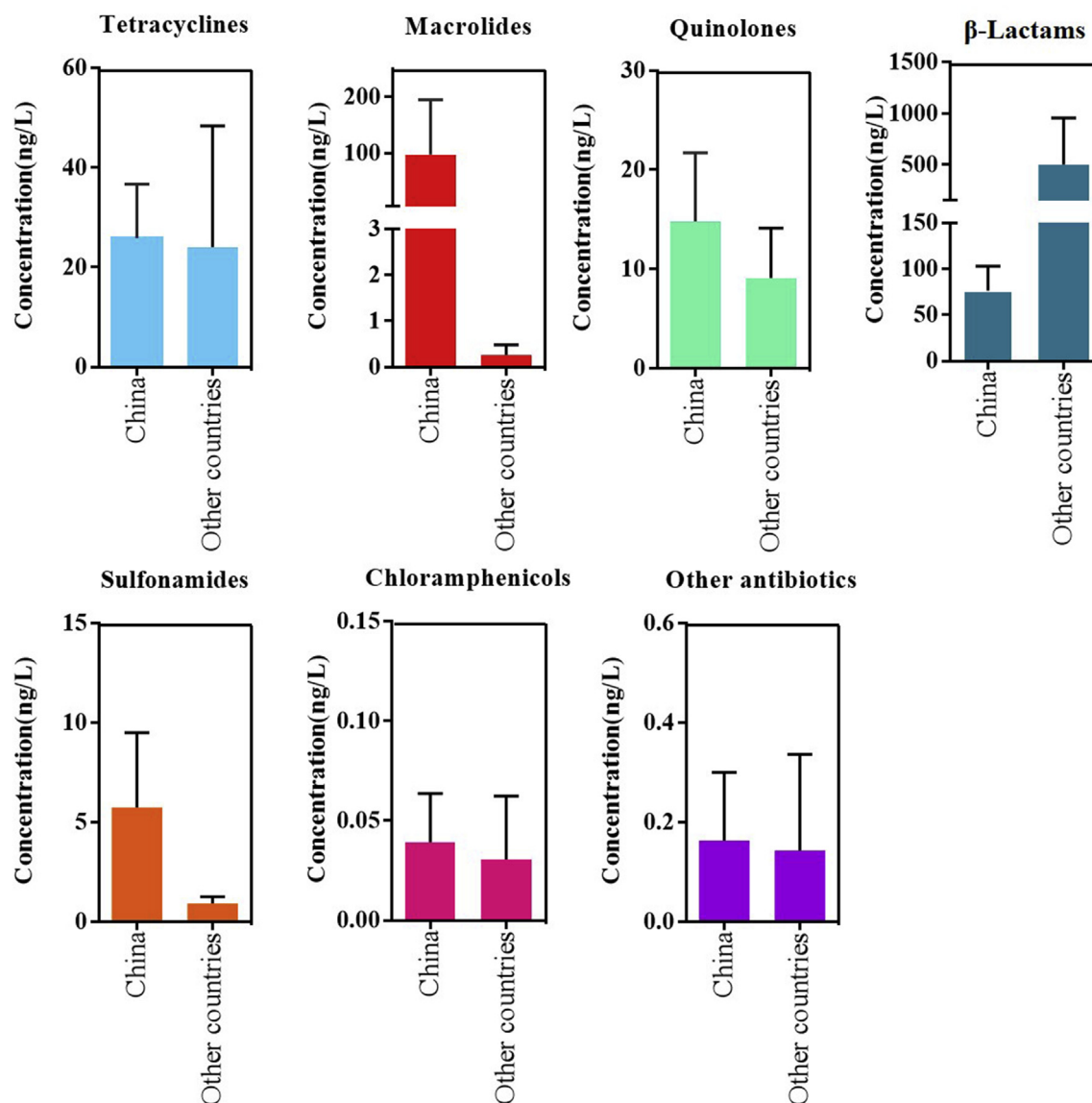


Fig. 3. Comparison of average antibiotic concentrations with 95% confidence interval in the collected Chinese and foreign brands of bottled drinking water in the Chinese market.

human microbiome, but a long-term exposure to such a mixture of dozens to hundreds of antibiotic residues may play a more powerful and far-reaching role in the selection of bacterial resistance than the antibiotics used in medical treatment. However, the mixture toxicities of antibiotics to the overall gastrointestinal bacteria, in particular long-term exposure to antibiotic mixtures at the residual levels and their corresponding metabolites, has not yet been well understood (Rodríguez-Mozaz and Weinberg, 2010). Therefore, the information and data required to quantify the risks of a mixture of antibiotic residues in the environment are severely limited. Therefore, the exposure risks from residual antibiotics in different types of drinking water were assessed individually in the present study based on the available toxicity information and data.

Sixteen analytes were detectable in more than 30% of the filtered tap water, the Chinese brands of bottled water and the foreign brands of bottled water samples. These 16 analytes are a potential list of antibiotic residues to be recommended for monitoring in drinking water. The estimated average and median daily intake ranged from 0.0058 and 0 ng/kg/day for sulfamethoxazole to 2.3 and 0.54 ng/kg/day for dicloxacillin in scenario 1, from 0.00077

and 0 ng/kg/day for monensin to 2.0 and 0.14 ng/kg/day for roxithromycin in scenario 2, and from 0.00074 and 0.00030 ng/kg/day for monensin to 14 and 2.7 ng/kg/day for dicloxacillin in scenario 3, respectively (Table 2).

For assessing human health risk when considering the detected antibiotics individually, both acceptable daily intakes (ADIs) derived from toxicological data and minimum inhibition concentrations (MICs) determined by microbiological approaches were adopted (Table 2). Based on the toxicological approach, no observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) were divided by uncertainty factors (UFs) to be converted to human equivalent doses for noncarcinogenic risk (Bruce et al., 2010; Dong et al., 2015). For norfloxacin with evidence of carcinogenicity in rats, the virtually safe dose, which corresponds to a cancer risk of one in a million, was calculated by dividing the maximum tolerated dose (MTD) by 740,000 to predict the cancer potential (Bruce et al., 2010; Gaylor and Gold, 1998). The toxicological ADIs were generally 10^2 – 10^8 times higher than the drinking daily intake of each individual antibiotic (Table 2), indicating that the intake of antibiotics via the three types of drinking water posed

Table 2

Daily intake of antibiotics by drinking different drinking waters and toxicological data.

Antibiotics	Average Drinking Intake (ng/kg/day) ^a			Toxicological ADI Point of Departure	Microbiological ADI		
	Scenario 1	Scenario 2	Scenario 3		ADI (µg/kg/day)	MIC ₅₀ ^e (µg/mL)	ADI (µg/kg/day)
Sulfamethazine	0.010 (0.083)	0.0057 (0.052)	0.0036 (0.016)	Embryo/fetal development (teratogenic) effects in rat, NOAEL = 545 mg/kg/day (Zoetis Inc.), UF = 300 (10,3,1,3,3) ^b	1800	1 (<i>Staphylococcus aureus</i>) (De Oliveira et al., 2000)	1.7
Doxycycline	0.10 (1.1)		0.15 (4.4)	Developmental effects in women exposed during pregnancy, LOAEL = 3.0 mg/kg/day (Snyder et al., 2010), UF = 300 (1,3,10,3,3)	10	2 (<i>Peptostreptococcus</i>) (Toxicity information for tetracycline, 2019)	3.4
Ciprofloxacin	0.015 (0.15)	0.030 (0.22)	0.028 (0.077)	Cartilage injury, NOAEL = 10 mg/kg/day (Bradley and Jackson, 2011), UF = 10000 (10,10,1, 10,10)	1.0	0.008 (<i>E. coli</i>) (Jeong et al., 2009)	0.014
Enoxacin	0.062 (0.81)	0.027 (0.16)	0.031 (0.16)	N.A.	N.A.	6.3 (<i>Streptococcus</i>) (Chin and Neu, 1983)	10
Sulfamethoxazole	0.0058 (0.026)	0.082 (1.3)		Embryo/fetal development effects in Rat, NOAEL = 512 mg/kg/day (Pfizer, 2018), UF = 300 (10,3,1,3,3)	1700	32 (<i>Peptococcus</i> and <i>Peptostreptococcus</i> spp.) (Wust and Wilkins, 1978)	54
Monensin	0.015 (0.25)	0.00077 (0.0088)	0.00074 (0.012)	Maternal toxicity and teratogenicity in pregnant rabbits, NOAEL = 0.76 mg/kg/day (U.S. FDA, 2019), UF = 300 (10,3,1,3,3)	2.5	0.5 (<i>Peptostreptococcus</i>) (JECFA, 2006)	0.85
Clarithromycin	0.012 (0.23)	0.017 (0.083)		NA	NA	0.5 (<i>Peptostreptococcus</i> spp.) (Citron and Appleman, 2001)	0.85
Enrofloxacin	0.020 (0.34)	0.010 (0.098)	0.010 (0.063)	Subchronic oral toxicity study in dogs, NOAEL = 3 mg/kg bw/day (Zhou, 2019), UF = 10000 (10,10,1,10,10)	0.3	0.062 (<i>E. coli</i>) (Committee for veterinary, 2019)	0.10
Roxithromycin	0.037 (0.69)	2.0 (36)		NA	NA	1 (<i>Peptostreptococcus</i> spp.) (Citron and Appleman, 2001)	1.7
Chlortetracycline	0.051 (0.35)	0.23 (1.7)	0.038 (0.24)	Generation reproductive toxicity in rat (Oral), NOAEL = 500 mg/kg/day (Zoetis Inc.), UF = 1000 (10, 3, 1, 10, 3)	500	16 (<i>M. hyopneumoniae</i>) (Jang et al., 2016)	27
Dicloxacinil	2.3 (29)	0.85 (4.5)	14 (91)	NA	NA	8 (<i>Streptococcus</i>) (Yu et al., 2017)	14
Tetracycline	0.061 (0.83)	0.072 (1.5)	0.10 (2.0)	Developmental toxicity in humans, LOAEL = 17 mg/kg/day (COT, 2014), UF = 100 (1,3,10,1,3)	170	2 (<i>Peptostreptococcus</i> spp.) (Wouters et al., 2019)	3.4
4-epitetracycline	0.026 (0.44)	0.034 (0.62)	0.035 (0.85)	NA	NA	NA	NA
Oxytetracycline	0.37 (15)	0.22 (2.4)	0.066 (0.16)	Embryo/fetal development effects in rat (oral), NOAEL = 100 mg/kg/day (Zoetis Inc.), UF = 1000 (10, 3, 1, 10,3)	100	4 (<i>Peptostreptococcus</i> spp.) (Wouters et al., 2019)	6.8
Levofloxacin	0.028 (0.36)	0.068 (0.61)	0.015 (0.036)	Levofloxacin: cartilage injury, NOAEL = 3 mg/kg/day (Bradley and Jackson, 2011), UF = 10000 (10, 10, 1, 10, 10)	0.30	0.094 (<i>K. pneumoniae</i>) (Grillon et al., 2016)	0.16
Ofloxacin	0.22 (7.8)	0.14 (1.5)	0.091 (0.43)	Evidence of carcinogenicity in rats, MTD = 18 mg/kg/day (Food Safety Commission of Japan (Food Safety Commission of Japan FSCJ, 2019). ^c	0.024 ^d	1.6 (<i>Streptococcus</i>) (Chin and Neu, 1983)	2.7
Norfloxacin							
Trimethoprim	0.024 (0.14)	0.026 (0.31)	0.0086 (0.020)	Embryo/fetal development effects in rabbit, NOAEL = 30 mg/kg/day (Pfizer, 2018), UF = 300 (10,3,1,3,3)	100	0.3 (<i>Peptococcus</i> and <i>Peptostreptococcus</i> spp.) (Wust and Wilkins, 1978)	0.51

^a The maximum daily intake is shown in parentheses for each compound.^b UF₁(UF₁,UF₂,UF₃,UF₄,UF₅). UF₁: interspecies difference 10; UF₂: intraspecies difference (10 or 3 when the toxicology experiment was based on a sensitive population), UF₃: extrapolation factor from LOAEL to NOAEL (10); UF₄: extrapolation factor from a subchronic to a chronic study (10 or 3 when the toxicology endpoint was reproductive or developmental toxicity); UF₅: database uncertainties (10, or 3 when the database including either a prenatal study or a 2-generation reproductive study).^c MTD: maximum tolerated dose.^d Virtually safe dose.^e Types of bacteria in parentheses.

no immediate human development toxicity and threat of cartilage injury.

The toxicological ADI is mainly applicable to assessing the risk of development toxicity and reproduction toxicity. However, the most likely risk associated with antibiotic residues in drinking water would be promoting emergence and selection for resistant commensal bacteria inhabiting the human body, especially the intestinal flora. To estimate the risk of resistance selection as a result of exposure to the residual antibiotic concentrations in drinking water, minimal selective concentrations (MSCs) for antibiotic resistance selection should be used to derive the microbiological ADI. Minimal selective concentration is initially defined as the lowest antibiotic concentration that could give a competitive advantage in growth rate to a resistant genotype over the susceptible genotype strain (Sandegren, 2014), and currently selective concentrations of antibiotics in complex communities have been gradually assessed in recent studies. (Kraupner et al., 2018; Lundstrom et al., 2016; Murray et al., 2018). Nevertheless, studies

on MSCs are scarce to date, and there is little to no available MSC data derived from the human microbial community. Minimum inhibition concentrations were used to predict MSCs by dividing by a factor of 10 based on the median MIC/MSC ratio (Gullberg et al., 2011). Then, the microbiological ADI was calculated using the following equation (Bruce et al., 2010; Jeong et al., 2009; Leung et al., 2013; Schwab et al., 2005):

$$ADI = \frac{MIC_{50} \times MCC}{10 \times EF \times BW} \quad (3)$$

where MIC₅₀ is the concentration inhibiting 50% of the human commensal microflora, which generally includes *Escherichia coli* (*E. coli*), *Peptostreptococcus*, *Streptococcus*, and *Klebsiella pneumoniae*; 10 is the extrapolation factor from MIC to MSC (Gullberg et al., 2011); MCC refers to the mass of colonic content (220 g); and EF is the effective fraction of the oral dose available to the colonic microorganisms (20%) (Bruce et al., 2010; Leung et al., 2013). The

microbiological ADIs were generally 10^2 – 10^6 times higher than the drinking daily intake for most of the detected compounds (Table 2), indicating that the exposure to individual residual antibiotics in drinking water may have no significant effects on human gut bacteria.

Although all the detected antibiotics may pose a low risk to human health when considered individually at the residual level in drinking water, there were still many uncertainties in the aforementioned risk assessment. First, no data are available on the gastrointestinal absorption of daily intake of different antibiotics residuals in food and drinking water. The default value of 20% for different antibiotics is highly uncertain. Second, it has become increasingly clear that long-term antibiotic exposure could contribute to the evolution of high-level bacterial resistance at concentrations that are several hundred-fold below MICs (Wistrand-Yuen et al., 2018). The use of a factor of 10 to extrapolate MSCs may overestimate the value of the microbiological ADI and underestimate health risk. Third, the metabolism of xenobiotic antibiotics by different gut bacterial species could be significantly perturbed in a species-specific manner (Zhang et al., 2018). It was very likely that the MSC values of different gut bacteria vary greatly and that the use of an MSC of a certain strain would lead to neglecting the selection of sensitive gut bacteria. Last, the metabolites produced from the gastrointestinally absorbed antibiotics by gut bacteria may pose even greater toxicity to human health than the original antibiotics. Nonetheless, little is known about the possible interactions and metabolism of the gastrointestinally absorbed antibiotics in the highly heterogeneous gastrointestinal system due to the technical challenges of analyzing the unknown metabolites. All of these issues collectively indicate that further research is urgently needed to develop a more comprehensive assessment of the overall human exposure to antibiotic residues in the environment, MSCs in complex microbiome communities in the human body, and the metabolism of xenobiotic antibiotics in the gastrointestinal system.

4. Conclusions

The study is the first to survey approximately 100 antibiotics in different types of drinking water in China. The screening analysis provides cost- and time-effective monitoring a wider range of antibiotic residues in drinking water. The results suggested a complex antibiotic pollution in Chinese drinking water, with a prevalence of macrolides. Based on the levels of antibiotic residues analyzed in different types of drinking water, the individual and overall values of daily intake of all the detected antibiotics were estimated for three scenarios: only filtered tap water was drunk, only Chinese brands of bottled water was drunk, and only foreign brands of bottled water was drunk. Risk of human exposure to trace antibiotics via drinking water was assessed individually, indicating that the individual antibiotic residues in drinking water may pose low risk to human health. However, the data and knowledge required to comprehensively characterize the overall risks of antibiotic residues exposure is severely limited. Further research is needed for a more in-depth understanding of the potential adverse effects of exposure to environmental antibiotic residues on the human microbiome.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Financial support from the National Natural Science Foundation of China (Grant Nos. 21806071 and 41890852) and the Leading Talents of Guangdong Province Program is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.watres.2020.115699>.

References

- Ben, Y., Fu, C., Hu, M., Liu, L., Wong, M.H., Zheng, C., 2019. Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: a review. *Environ. Res.* 169, 483–493.
- Benotti, M.J., Trenholm, R.A., Vanderford, B.J., Holady, J.C., Stanford, B.D., Snyder, S.A., 2009. Pharmaceuticals and endocrine disrupting compounds in US drinking water. *Environ. Sci. Technol.* 43 (3), 597–603.
- Blaser, M.J., 2016. Antibiotic use and its consequences for the normal microbiome. *Science* 352 (6285), 544–545.
- Boleda, M.R., Alechaga, E., Moyano, E., Galceran, M.T., Ventura, F., 2014. Survey of the occurrence of pharmaceuticals in Spanish finished drinking waters. *Environ. Sci. Pollut. Res.* 21 (18), 10917–10939.
- Borecka, M., Bialk-Bielińska, A., Siedlewicz, G., Stepnowski, P., Pazdro, K., 2014. The influence of matrix effects on trace analysis of pharmaceutical residues in aqueous environmental samples. Insights on environmental changes. Series: GeoPlanet: earth and planetary Sciences. In: Tymon Zielinski, Ksenia Pazdro, Agata Dragan-Górska and Agata Weydmann. Springer International Publishing, Cham, ISBN 978-3-319-03682-3, pp. 1–16, 14(1), 1–16.
- Bradley, J.S., Jackson, M.A., Comm Infect, D., 2011. The use of systemic and topical fluoroquinolones. *Pediatrics* 128 (4), E1034–E1045.
- Bruce, G.M., Pleus, R.C., Snyder, S.A., 2010. Toxicological relevance of pharmaceuticals in drinking water. *Environ. Sci. Technol.* 44 (14), 5619–5626.
- Cai, M.-Q., Wang, R., Feng, L., Zhang, L.-Q., 2015. Determination of selected pharmaceuticals in tap water and drinking water treatment plant by high-performance liquid chromatography-triple quadrupole mass spectrometer in Beijing, China. *Environ. Sci. Pollut. Res.* 22 (3), 1854–1867.
- Citron, D.M., Appleman, M.D., 2001. Comparative in vitro activities of ABT-773 against 362 clinical isolates of anaerobic bacteria. *J. Antimicrob. Chemother.* 45 (1), 345–348.
- Committee for veterinary medicinal products: enrofloxacin, summary report. <http://www.emea.europa.eu/pdfs/vet/mrls/enro01en.pdf> (accessed date: 5 October, 2019).
- Chin, N.X., Neu, H.C., 1983. In vitro activity of enoxacin, a quinolone carboxylic-acid, compared with those of norfloxacin, new beta-lactams, aminoglycosides, and trimethoprim. *J. Antimicrob. Chemother.* 24 (5), 754–763.
- Damman, C.J., Miller, S.I., Surawicz, C.M., Zisman, T.L., 2012. The microbiome and inflammatory bowel disease: is there a therapeutic role for fecal microbiota transplantation? *Am. J. Gastroenterol.* 107 (10), 1452.
- De Oliveira, A.P., Watts, J.L., Salmon, S.A., Aerestrup, F.M., 2000. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Europe and the United States. *J. Dairy Sci.* 83 (4), 855–862.
- Díaz-Cruz, M.S., García-Galán, M.J., Damià, B., 2008. Highly sensitive simultaneous determination of sulfonamide antibiotics and one metabolite in environmental waters by liquid chromatography–quadrupole linear ion trap–mass spectrometry. *J. Chromatogr. A* 1193 (1–2), 50–59.
- Dong, Z., Liu, Y., Duan, L., Bekele, D., Naidu, R., 2015. Uncertainties in human health risk assessment of environmental contaminants: a review and perspective. *Environ. Int.* 85, 120–132.
- Food Safety Commission of Japan (FSCJ), 2019. Risk assessment report for norfloxacin. https://www.fscj.go.jp/english/evaluationreports/vetmedicine/norfloxacin_fs60.pdf (accessed date: 5 October, 2019).
- Gaylor, D.W., Gold, L.S., 1998. Regulatory cancer risk assessment based on a quick estimate of a benchmark dose derived from the maximum tolerated dose. *Regul. Toxicol. Pharmacol.* 28 (3), 222–225.
- Grillon, A., Schramm, F., Kleinberg, M., Jehl, F., 2016. Comparative activity of ciprofloxacin, levofloxacin and moxifloxacin against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* assessed by minimum inhibitory concentrations and time-kill studies. *PLoS One* 11 (6).
- Gullberg, E., Cao, S., Berg, O.G., Ilback, C., Sandegren, L., Hughes, D., Andersson, D.I., 2011. Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathog.* 7 (7).
- Hanna, N., Sun, P., Sun, Q., Li, X., Yang, X., Ji, X., Zou, H., Ottoson, J., Nilsson, L.E., Berglund, B., Dyar, O.J., Tamhankar, A.J., Lundborg, C.S., 2018. Presence of antibiotic residues in various environmental compartments of Shandong province in eastern China: its potential for resistance development and ecological and human risk. *Environ. Int.* 114, 131–142.
- Hirsch, R., Ternes, T., Haberer, K., Kratz, K.-L., 1999. Occurrence of antibiotics in the

- aquatic environment. *Sci. Total Environ.* 225, 109–118.
- Jang, J., Kim, K., Park, S., Park, B., Um, H., Coulier, M., Hahn, T.-W., 2016. In vitro antibiotic susceptibility of field isolates of *Mycoplasma hyopneumoniae* and *Mycoplasma hyorhinis* from Korea. *Korean J. Vet. Res.* 56 (2), 109–111.
- Jeong, S.-H., Song, Y.-K., Cho, J.-H., 2009. Risk assessment of ciprofloxacin, flavomycin, olaquinox and colistin sulfate based on microbiological impact on human gut biota. *Regul. Toxicol. Pharmacol.* 53 (3), 209–216.
- Ji, K., Kho, Y., Park, C., Paek, D., Ryu, P., Paek, D., Kim, M., Kim, P., Choi, K., 2010. Influence of water and food consumption on inadvertent antibiotics intake among general population. *Environ. Res.* 110 (7), 641–649.
- Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2006. Evaluation of Certain Veterinary Drug Residues in Food; 66th Report of the Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Committee on Food Additives. World Health Organization. WHO Technical Report Series, 939.
- Kraupner, N., Ebmeyer, S., Bengtsson-Palme, J., Fick, J., Kristiansson, E., Flach, C.-F., Larsson, D.G.J., 2018. Selective concentration for ciprofloxacin resistance in *Escherichia coli* grown in complex aquatic bacterial biofilms. *Environ. Res.* 116, 255–268.
- Kumar, R.R., Lee, J.T., Cho, J.Y., 2012. Fate, occurrence, and toxicity of veterinary antibiotics in environment. *J. Korean Soc. Appl. Biol. Chem.* 55 (6), 701–709.
- Le Page, G., Gunnarsson, L., Snape, J., Tyler, C.R., 2017. Integrating human and environmental health in antibiotic risk assessment: a critical analysis of protection goals, species sensitivity and antimicrobial resistance. *Environ. Int.* 109, 155–169.
- Leung, H.W., Jin, L., Wei, S., Tsui, M.M.P., Zhou, B., Jiao, L., Cheung, P.C., Chun, Y.K., Murphy, M.B., Lam, P.K.S., 2013. Pharmaceuticals in tap water: human health risk assessment and proposed monitoring framework in China. *Environ. Health Perspect.* 121 (7), 839–846.
- Li, N., Ho, K.W.K., Ying, G.-G., Deng, W.-J., 2017. Veterinary antibiotics in food, drinking water, and the urine of preschool children in Hong Kong. *Environ. Int.* 108, 246–252.
- Lundstrom, S.V., Ostman, M., Bengtsson-Palme, J., Rutgersson, C., Thoudal, M., Sircar, T., Blanck, H., Eriksson, K.M., Tysklind, M., Flach, C.-F., Larsson, D.G.J., 2016. Minimal selective concentrations of tetracycline in complex aquatic bacterial biofilms. *Sci. Total Environ.* 553, 587–595.
- Ministry of agriculture of the people Republic of China, 2011. Announcement No. 193. Available. http://jiuban.moa.gov.cn/zwlml/tzgg/gg/201104/t20110422_1976324.htm. accessed: 11/02/2020.
- Murray, A.K., Zhang, L., Yin, X., Zhang, T., Buckling, A., Snape, J., Gaze, W.H., 2018. Novel insights into selection for antibiotic resistance in complex microbial communities. *mBio* 9 (4).
- National Academies of Sciences, E., Medicine, 2018. Environmental Chemicals, the Human Microbiome, and Health Risk: A Research Strategy. National Academies Press.
- Pérez-Cobas, A.E., Gosálbes, M.J., Friedrichs, A., Knecht, H., Artacho, A., Eismann, K., Otto, W., Rojo, D., Bargiela, R., von Bergen, M., 2012. Gut microbiota disturbance during antibiotic therapy: a multi-omic approach. *Gut* 62 (11), 1591–1601.
- Perret, D., Gentili, A., Marchese, S., Greco, A., Curini, R., 2006. Sulphonamide residues in Italian surface and drinking waters: a small scale reconnaissance. *Chromatographia* 63 (5–6), 225–232.
- Pfizer, Inc. Safety data sheet for trimethoprim and sulfamethoxazole tablets. https://www.pfizer.com/sites/default/files/products/material_safety_data/trimethoprim_sulfamethoxazole_tabs_21-aug-2018.pdf accessed date: 5 October, 2019.
- Qiao, M., Ying, G.-G., Singer, A.C., Zhu, Y.-G., 2017. Review of antibiotic resistance in China and its environment. *Environ. Int.* 110, 160–172.
- Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (RCEESAS), 2018. Research Report of Major Science and Technology Program for Water Pollution Control and Treatment of China (2014–2017): Source Water Quality Characteristics in Key Basin and Safe Drinking Water Strategy.
- Rodriguez-Mozaz, S., Weinberg, H.S., 2010. Meeting report: pharmaceuticals in water-an interdisciplinary approach to a public health challenge. *Environ. Health Perspect.* 118 (7), 1016–1020.
- Sandberg, A., Jensen, K.S., Baudoux, P., Van Bambeke, F., Tulkens, P.M., Frimodt-Moller, N., 2010. Intra- and extracellular activities of dicloxacillin against *Staphylococcus aureus* in vivo and in vitro. *Antimicrob. Agents Chemother.* 54 (6), 2391–2400.
- Sandegren, L., 2014. Selection of antibiotic resistance at very low antibiotic concentrations. *Ups. J. Med. Sci.* 119 (2), 103–107.
- Schwab, B.W., Hayes, E.P., Fiori, J.M., Mastrocco, F.J., Roden, N.M., Cragin, D., Meyerhoff, R.D., D'Aco, V.J., Anderson, P.D., 2005. Human pharmaceuticals in US surface waters: a human health risk assessment. *Regul. Toxicol. Pharmacol.* 42 (3), 296–312.
- Simazaki, D., Kubota, R., Suzuki, T., Akiba, M., Nishimura, T., Kunikane, S., 2015. Occurrence of selected pharmaceuticals at drinking water purification plants in Japan and implications for human health. *Water Res.* 76, 187–200.
- The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), Assessment of the adequacy of the 10-fold uncertainty factor to allow for interspecies variation in developmental toxicity. <https://cot.food.gov.uk/sites/default/files/cot/tox201417.pdf> (accessed date: 5 October, 2019).
- Toxicity information for tetracycline. <http://www.antimicrobe.org/d32tab.htm> (accessed date: 5 October, 2019).
- Snyder, S.A., Stanford, B.D., Bruce, G.M., Pleus, R.C., drewes, J.E., 2010. Identifying Hormonally Active Compounds, Pharmaceuticals, and Personal Care Product Ingredients of Health Concern from Potential Presence in Water Intended for Indirect Potable Reuse (WRF Project 05-005). WaterReuse Foundation, Alexandria, VA, p. 228.
- U.S. Food and Drug Administration (U.S.FDA). Freedom of information summary for monensin. <https://www.fda.gov/media/98126/download> accessed date: 5 October, 2019.
- Wang, H., Wang, B., Zhao, Q., Zhao, Y., Fu, C., Feng, X., Wang, N., Su, M., Tang, C., Jiang, F., 2015. Antibiotic body burden of Chinese school children: a multisite biomonitoring-based study. *Environ. Sci. Technol.* 49 (8), 5070–5079.
- Wang, H., Wang, N., Wang, B., Zhao, Q., Fang, H., Fu, C., Tang, C., Jiang, F., Zhou, Y., Chen, Y., Jiang, Q., 2016. Antibiotics in drinking water in Shanghai and their contribution to antibiotic exposure of school children. *Environ. Sci. Technol.* 50 (5), 2692–2699.
- Watkinson, A.J., Murby, E.J., Kolpin, D.W., Costanzo, S.D., 2009. The occurrence of antibiotics in an urban watershed: from wastewater to drinking water. *Sci. Total Environ.* 407 (8), 2711–2723.
- WHO (World Health Organization), 2018. WHO Report on Surveillance of Antibiotic Consumption: 2016–2018 Early Implementation. WHO Press, Geneva. Available at. <https://apps.who.int/iris/bitstream/handle/10665/277359/9789241514880-eng.pdf>. accessed: 11/02/2020.
- Wistrand-Yuen, E., Knopp, M., Hjort, K., Koskineniemi, S., Berg, O.G., Andersson, D.I., 2018. Evolution of high-level resistance during low-level antibiotic exposure. *Nat. Commun.* 9.
- Wouters, M.F.A., van Koten-Vermeulen, J.E.M., van Leeuwen, F.X.R., 2019. Toxicity data for chlortetracycline and tetracycline. <http://www.inchem.org/documents/jecfa/jecmono/v36je06.htm> accessed date: 5 October, 2019.
- Wust, J., Wilkins, T.D., 1978. Susceptibility of anaerobic bacteria to sulfamethoxazole-trimethoprim and routine susceptibility testing. *J. Antimicrob. Chemother.* 14 (3), 384–390.
- Xu, W., Zhang, G., Li, X., Zou, S., Li, P., Hu, Z., Li, J., 2007. Occurrence and elimination of antibiotics at four sewage treatment plants in the Pearl River Delta (PRD), South China. *Water Res.* 41 (19), 4526–4534.
- Yiruhan, Wang, Q.-J., Mo, C.-H., Li, Y.-W., Gao, P., Tai, Y.-P., Zhang, Y., Ruan, Z.-L., Xu, J.-W., 2010. Determination of four fluoroquinolone antibiotics in tap water in Guangzhou and Macao. *Environ. Pollut.* 158 (7), 2350–2358.
- Yu, W., Ji, J., Xiao, T., Ying, C., Fang, J., Shen, P., Xiao, Y., 2017. Determining optimal dosing regimen of oral administration of dicloxacillin using Monte Carlo simulation. *Drug Des. Dev. Ther.* 11, 1951–1956.
- Zhang, Q.-Q., Ying, G.-G., Pan, C.-G., Liu, Y.-S., Zhao, J.-L., 2015. Comprehensive evaluation of antibiotics emission and fate in the river basins of China: source analysis, multimedia modeling, and linkage to bacterial resistance. *Environ. Sci. Technol.* 49 (11), 6772–6782.
- Zhang, Y., Keerthisinghe, T.P., Han, Y., Liu, M., Wanjaya, E.R., Fang, M., 2018. Cocktail" of xenobiotics at human relevant levels reshapes the gut bacterial metabolome in a species-specific manner. *Environ. Sci. Technol.* 52 (19), 11402–11410.
- Zhang, S., Kang, Q., Peng, H., Ding, M., Zhao, F., Zhou, Y., Dong, Z., Zhang, H., Yang, M., Tao, S., Hu, J., 2019. Relationship between perfluorooctanoate and perfluorooctane sulfonate blood concentrations in the general population and routine drinking water exposure. *Environ. Int.* 126, 54–60.
- Zhou, T. Human food safety of new animal drugs: toxicology assessment. <https://www.accessdata.fda.gov/static/cvm/zhoutoxicologyAssessmentUpdated.pptx> (accessed date: 5 October, 2019).
- Zoetis Inc., Safty data sheet for Aureomycin® 90G. https://www.zoetis.com/contact/pages/product_information/msds_pi/msds/Aureomycin_90G.pdf (accessed date: 5 October, 2019).
- Zoetis Inc., Safty data sheet for Neo-sulfalyte bolus. <https://www.zoetis.ca/sds/technical-documents/neo-sulfalyte-canadian-english-sds.pdf> (accessed date: 5 October, 2019).
- Zuccato, E., Calamari, D., Natangelo, M., Fanelli, R., 2000. Presence of therapeutic drugs in the environment. *Lancet* 355 (9217), 1789–1790.