Science of the Total Environment 599–600 (2017) 298–304

Contents lists available at ScienceDirect

Science of the Total Environment

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

Antibiotics in a general population: Relations with gender, body mass index (BMI) and age and their human health risks

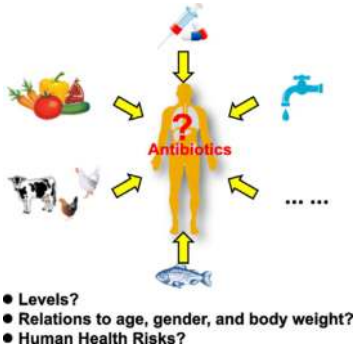
Sisi Liu [a], Guodong Zhao [b], Hongxia Zhao [a,*], Guangshu Zhai [c], Jingwen Chen [a], Haidong Zhao [b,*]

[a] Key Laboratory of Industrial Ecology and Environmental Engineering (Ministry of Education), School of Environmental Science and Technology, Dalian University of Technology, Linggong Road 2, Dalian 116023, China
[b] Gland surgery, The Second Affiliated Hospital of Dalian Medical University, Zhongshan Road 467, Dalian 116020, China
[c] 3M Environment, Health, Safety and Sustainability, 3M Center, Building 026-05-N-17, St. Paul, MN 55144-1000, USA

HIGHLIGHTS

- Serum levels of 40 antibiotics were determined in 107 normal adults.
- 28 antibiotics were detected and 5 were above 1000 ng/mL in 3.7% of the samples.
- Males had higher levels of the veterinary antibiotics than females ($p < 0.05$).
- There were significant BMI-related and age-related differences for sulfonamides.
- No health risks occurred except for azithromycin in one sample.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 10 March 2017
Received in revised form 26 April 2017
Accepted 27 April 2017
Available online xxxx

Editor: Jay Gan

Keywords:
Antibiotics
Gender
Age
Body mass index (BMI)
Human health risks

ABSTRACT

Recently, increasing regulatory and public attention has been paid to the exposure risks of antibiotics due to their occurrence and antibiotic resistance worldwide. However, limited information on antibiotic levels in general populations is available. Forty antibiotics, including 9 sulfonamides, 5 fluoroquinolones, 4 macrolides, 4 tetracyclines, 3 chloramphenicols, 12 β-lactams and 3 others, were analyzed in 107 serum samples of normal adults collected from a hospital in Dalian, North China, between 2015 and 2016 using solid-phase extraction (SPE) coupled with HPLC-MS/MS. The results clearly showed that antibiotics were present in the serum of these adults. Specifically, 28 antibiotics were detected in the samples, with detection frequencies ranging from 0.9% to 17.8%. The total antibiotic concentrations in 26.2% of the serum samples were between the LOD and 20.0 ng/mL. Importantly, the maximum concentrations of 5 antibiotics (trimethoprim, ciprofloxacin, cefaclor, lincomycin and erythromycin) were above 1000 ng/mL in 3.7% of the samples. Furthermore, the detection frequencies of 5 veterinary antibiotics, 7 human antibiotics and 16 human/veterinary antibiotics in the serum samples were 23.4%, 17.8% and 29.0%, respectively. Significant differences of the veterinary antibiotics between female and male adults and of the sulfonamides between different BMI (body mass index) groups were observed ($p < 0.05$). The concentrations of sulfonamides in elderly individuals were significantly higher ($p < 0.05$) than those in young people. Finally, our results showed that almost all of the adults had no health risks related to exposure to antibiotics at such levels despite the high effect ratio ($ER = 1.74$) for azithromycin in one sample. This study is the first to report the current status of antibiotics in human blood, which can help in better understanding the long-term effects of antibiotics on general populations and in identifying susceptible populations that are at high risk to antibiotic exposure.

* Corresponding authors.
E-mail addresses: hxzhao@dlut.edu.cn (H. Zhao), haidong@dlmedu.edu.cn (H. Zhao).

http://dx.doi.org/10.1016/j.scitotenv.2017.04.216
0048-9697/

1. Introduction

Antibiotics, as a class of pharmaceuticals, are extensively used in humans and animals for disease prevention, treatment and growth promotion (Kemper, 2008; Nathan and Cars, 2014). A high percentage of antibiotics or their metabolites are excreted from the bodies of humans and animals (Bound and Voulvoulis, 2004). These antibiotics are released into the environment via wastewater treatment plants (WWTPs) (Kim et al., 2005; Li and Zhang, 2013) and are thus frequently detected in the surroundings such as in waters, soils and sediments (Gao et al., 2012; Topp et al., 2016). Owing to their high use among human populations, their continuous introduction makes antibiotics "pseudo-persistent" in the natural environment (Gavalchin and Katz, 1994; Hektoen et al., 1995; Kemper, 2008).

Antibiotics are often abused and overused in animal husbandry and aquaculture (Lam et al., 2013; Liu and Wong, 2013), which leads to antibiotic residues in human foods. To date, over 70 antibiotics have been identified in various foods of animal origin, including chicken, pork, beef, and fish products (Chen et al., 2015; He et al., 2012; Yamaguchi et al., 2015). Thus, humans can suffer from antibiotic exposure by dietary intake of these contaminated foods. Furthermore, antibiotics can enter into human bodies via drug abuse (Li et al., 2012), drinking water (Leung et al., 2013; Wang et al., 2016b), and the inhalation of contaminated dust (Hamscher et al., 2003). The issue of antibiotics has represented a risk to human health. For example, allergic reactions can be evoked by penicillin and cephalosporins and transferred to human bodies by oral or parenteral routes (Kemper, 2008). Cases of nephrotoxicity have been reported after the administration of sulfonamides (Wawruch et al., 2002). In addition, evidence has demonstrated that some antibiotics can cause adverse effects on human bodies in the long term, such as childhood obesity (Riley et al., 2013), tooth discoloration (Sanchez et al., 2004), and photobiological damage (Habif, 2006; Wang and Lin, 2012). In addition, antibiotics can lead to the formation of resistant genes of bacteria strains (Zhu et al., 2013), which may ultimately transfer to humans via food chains (Zhi et al., 2016). According to a report by the World Health Organization (WHO), there will be >1,000,000 deaths annually due to antibiotic resistance by 2050 (WHO, 2014). Therefore, the existence of antibiotics in humans is of great concern to assess the human health risks.

Several studies have demonstrated that antibiotics are present in the urine of Chinese children (Wang et al., 2015; Wang et al., 2016a; Wang et al., 2016b). For example, 18 antibiotics were detected in the urine of Chinese healthy children, and the concentrations of ampicillin reached >40,000 ng/mL in a recent study (Wang et al., 2015). Furthermore, a number of clinical studies have shown that the uptake, volume of distribution, elimination, and associated half-lives of antibiotics in human bodies can be affected by age and gender (Cox and Blaser, 2015; Mueller et al., 2006). In addition, human gut microbiota can be altered after antibiotic treatment, which may lead to a change of human metabolism and body weight (Mikkelsen et al., 2016). Thus, it is plausible that the antibiotic body burden is related to age, gender, and body weight. However, as important biomarkers of many contaminations in human bodies (Bosca, 2012), no information on antibiotic levels in human blood is currently available.

The main objective of the present study is to extensively examine the human body burden of antibiotics in serum from a population of 107 normal adults collected in Dalian, North China. Forty antibiotics (Table S1), including 9 sulfonamides (SAs), 5 fluoroquinolones (FQs), 4 macrolides (MLs), 4 tetracyclines (TCs), 3 chloramphenicols (CAPs), 12 β-lactams (10 cephalosporins (CFs), penicillin and amoxicillin) and 3 others (trimethoprim, rifampin and lincomycin), are analyzed using solid phase extraction (SPE) coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS). Furthermore, the relations of antibiotic concentrations with age, body mass index (BMI) and gender are explored in detail. Because gender, age and the BIM could be associated with the body burden of antibiotics, to exclude the possible effects of the other two factors (considered covariates), a multiple linear regression model was performed when analyzing the relation of antibiotic concentrations with one of these factors (Makey et al., 2016). Finally, the human health risks of these individuals related to antibiotic exposure are assessed using an improved model based on the "read-across" hypothesis, which presumes that a pharmacological effect can be observed only if the plasma concentration of a chemical is close to its human therapeutic plasma concentration (Rand-Weaver et al., 2013). This hypothesis is widely applied in assessing the potential risks of human pharmaceuticals in wildlife such as fish, which can greatly fill up data gaps of thousands of pharmaceuticals since it is unrealistic to assess each drug experimentally (Huggett et al., 2003). This pilot study reports for the first time the body burden of antibiotics in a Chinese general population, which can help understand and assess the human health risks of antibiotic exposure.

2. Materials and methods

2.1. Sampling

All blood samples were collected from 107 normal adults (55 females, ages: 25–85 years old) in a local hospital in Dalian from November 2015 to February 2016. All subjects participating in this study were volunteers who provided their informed written consent. These blood samples were collected at the same time of one day, place, and preservation conditions. Fasting blood in the morning was required. The biological indexes of the subjects (Table S2), including their age, gender, weight and height, were recorded in the hospital. Five milliliters of blood from a cubital vein were collected in a sterile serum tube (Sanli, China) without anticoagulant. The serum was separated by centrifugation at 4000 rpm for 5 min using a high speed centrifuge (Thermo Fisher Scientific, Waltham, MA, USA) and then transferred to another tube. All serum samples were kept at −20 °C until further analyzed.

2.2. Standards and reagents

Forty antibiotics, including 9 sulfonamides (sulfadiazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfadimidine, sulfamethoxazole, sulfadimethoxine, sulfamonomethoxine and sulfachlorpyridazine), 5 fluoroquinolones (enoxacin, ofloxacin, ciprofloxacin, norfloxacin and enrofloxacin), 4 macrolides (roxithromycin, erythromycin, azithromycin and clarithromycin), 12 β-lactams (ceftiofur, cephalonium, cefuroxime, cefapirin, cefalexin, cefquinome, cefepime, cefoperazone, cefazoline, cefaclor, penicillin and amoxicillin), 4 tetracyclines (tetracycline, oxytetracycline, chlortetracycline and doxycycline), 3 chloramphenicols (chloramphenicol, thiamphenicol and florfenicol) and 3 others (trimethoprim, rifampin and lincomycin), were purchased from the Dr. Ehrenstorfer Co., Ltd. (Germany). The purity of all of these standards was >98%. The labeled compounds, including sulfamethoxazole-D_4, atrazine-D_5, trimethoprim-D_3, ciprofloxacin-D_8, chloramphenicol-D_5, and caffeine-$^{15}N_2$, were also purchased from the Dr. Ehrenstorfer Co., Ltd. The stock solutions of 1.00 mg/mL for these standards were prepared in methanol and stored at −4 °C for 6 months.

Methanol and acetonitrile were of HPLC grade from the Tedia Inc. (Ohio, USA). Ammonium formate (analytical purity) was obtained from the Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Formic acid (analytical purity) was purchased from the Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Ammonium hydroxide (guaranteed purity) was purchased from the Guangfu Fine Chemical Research Institute (Tianjin, China). Ultrapure water (18.2 MΩ·cm, 25 °C) was obtained with an OKPURE water system (Laikie Instrument Co., Ltd., Shanghai, China).

2.3. Sample extraction

The sample extraction was performed by following the same procedure described by Kwok et al. (2010), with minor modification. Briefly, 1.0 mL of plasma specimens were spiked with 10 ng of surrogate standards (sulfamethoxazole-D_4, atrazine-D_5, trimethoprim-D_3, chloramphenicol-D_5, and ciprofloxacin-D_8), diluted with 1 mL of 1.0 M ammonium acetate (pH = 5) to denature the serum albumin, vortexed thoroughly and kept overnight. The mixture was transferred onto an Oasis HLB cartridge (60 mg, 3 mL; 30 μm) preconditioned with 2 mL of methanol and equilibrated with 2 mL of ultrapure water. The cartridge was sequentially washed with 4 mL of ultrapure water to remove matrix interferences and the ammonium acetate buffer. Afterwards, the cartridge was dried under vacuum for 30 min. The retained analytes and surrogates were eluted with 2 mL of methanol containing 5% ammonium hydroxide. The eluent was completely evaporated at 40 °C under a gentle stream of nitrogen. The dry residues were reconstituted with 200 μL of initial mobile phase (95% A: 5% B). Ten nanograms of caffeine-$^{15}N_2$ were added. Finally, the solution was filtered by 0.22 μm film and 10 μL of extracts were injected for LC/MS/MS analysis.

2.4. Instrument analysis

Chemical analysis was performed using an Agilent 1100 high performance liquid chromatography-tandem 6410B quadrupole mass spectrometry (LC-MS/MS) equipped with an electrospray ionization (ESI) source using the multiple reaction monitoring (MRM) mode. An XTerra® MS C18 column (2.1 mm × 100 mm, 3.5 μm; Waters, Milford, MA, USA) was used to separate the analytes, and the column temperature was 40 °C. The gradient elution program was initiated with 95% A (0.1% v/v formic acid with 15.9 mmol/L ammonium formate in water, pH = 2.4) and 5% B (methanol/acetonitrile, 1/1 in v/v) and followed by a linear segment with 5–88% A (0–30 min) at a flow rate of 0.3 mL/min. The MRM transitions and fragment voltages, collision energies, and retention times are shown in Table S3. Other MS conditions are: drying gas flow, 8 L/min; drying gas temperature, 350 °C; nebulizer pressure, 38 psi; capillary voltage, 4000 V; delta electron multiplier voltage, 400 V; and collision gas, N_2 at 1.75×10^7 Torr.

2.5. Quality assurance and quality control

An eight-point matrix-matched calibration curve from 0.5 to 2000 ng/mL was prepared for all target analytes. The regression coefficients (r^2) ranged from 0.979 to 0.999 (Table S4). The limits of detection (LODs) and limits of quantitation (LOQs) were defined as three times and ten times the standard deviation (SD) of the mean procedural blanks ($n = 6$), respectively. The LODs of the target antibiotics were 0.0163–1.81 ng/mL (Table S4). To prevent the carryover of analytes from sample to sample, a solvent blank was analyzed before each batch of 12 samples to check all appliances, equipment, and solvents. In addition, one procedural blank was analyzed within each batch to monitor the contaminants. All target antibiotics in blanks were below the LODs. As a check for instrumentation drift in the response factors, a midpoint calibration standard was injected after each batch. The accuracy and repeatability of the overall method were determined by the analysis of six replicates of blank matrices (i.e., 5% fetal calf serum) spiked at 10 and 100 ng/mL. The spiked recoveries of the target antibiotics ranged from 41.3% to 132% (Table S4). The relative standard deviations (RSDs) of the recoveries were <24.6%. All samples were spiked with the surrogate standards prior to extraction to assess the recoveries of the target compounds in the serum samples. The surrogate recoveries varied from 69.4% to 119%. To account for the potential loss of analytes during sample preparation, the matrix-induced signal suppression or enhancement in ionization, and the variation in the instrumental response, the concentrations of analytes were quantified with respect to the mass of the respective surrogate standards using the isotope dilution method. The instrumental standard (IS), caffeine-$^{15}N_2$, was used to compensate for the variation in the volumes of the final extracts and to check the instrument performance during the whole measurement series. That is, the peak area of the IS was monitored to detect problems in regard to instrumental sensitivity or the injection volume. If the IS area decreased significantly (i.e., a signal reduction of >20% within the same matrix), then the series was stopped, and the instrument was cleaned.

2.6. Health risk assessment of human antibiotic exposure

To assess the human health risks of these adults related to antibiotics, a risk model was improved based on the "read-across" hypothesis, which can be used to assess the potential impacts of human pharmaceuticals on fish in environmental toxicology (Huggett et al., 2003). In this improved model, an effect ratio (*ER*) value of an antibiotic can be derived from dividing its measured concentration in the blood of one person ($C_{measured}$) by its human therapeutic plasma concentration (H_TPC, Table S1) and applying an uncertainty factor (*UF*) of 10, which accounts for the inter-individual variability (e.g., gender, age, lifestyle, nutritional status, pharmacokinetics, and pharmacodynamics) in the general population with antibiotic exposure (Ginsberg et al., 2004; Renwick, 1998). It is stipulated that a drug will result in a pharmacological effect only if its plasma concentration reaches or exceeds the H_TPC value (Rand-Weaver et al., 2013). Thus, in the present study, we assumed that a high human health risk would occur when the *ER* value of an antibiotic for an adult was not <1.

$$ER = \frac{C_{measured}}{H_TPC \times UF}$$

2.7. Data analysis

Values below the LODs were treated as zero for the calculation of total concentrations, means, and medians (Thomas et al., 2006). However, when performing statistical analyses, those below the LODs were taken as 1/2 LODs. The data were log-transformed prior to conducting statistical tests. Student's *t*-test was used to compare the antibiotic concentrations in different groups (gender, body mass index (BMI), or age) if the data passed the normality test and presented equal variance. If the data were not normally distributed, the nonparametric Mann-Whitney *U* test was employed for intergroup comparisons. A multiple linear regression model was performed to adjust for potential effects on the relations of the serum concentrations with gender, age, and BMI. All these relations were analyzed based on a small population ($n = 44$) that excluded the undetected samples. The statistical analyses were conducted using SPSS 19.0 software (IBM Co, Armonk, NY, USA). The level of significance was set to $p < 0.05$.

3. Results and discussion

3.1. Frequencies and concentrations in serum

Twenty-eight antibiotics from eight categories were detected in the serum samples (Table 1, Fig. S1). The overall detection frequency of these antibiotics was 41.1%, and the detection frequencies of individual antibiotics ranged from 0.9% to 17.8%. Specifically, the dominant categories of detected antibiotics were SAs and FQs, with the detection frequencies of each antibiotic categories being 30.8% (SAs + trimethoprim), 25.2% (FQs), 7.5% (CFs), 6.5% (MLs), 2.8% (TCs), 2.8% (CAPs), 3.7% (lincomycin), and 1.9% (penicillin). Five antibiotics, including sulfamerazine (14.0%), sulfadimethoxine (13.1%), trimethoprim (10.3%), enrofloxacin (17.8%) and ofloxacin (13.1%), were detected in >10% of the serum samples. Among these detected antibiotics, five

Table 1
Selected percentile concentrations, maximum concentrations (ng/mL) and detection frequencies (%) of antibiotics in the serum samples ($n = 107$) collected from Dalian, China.

Antibiotic	Percentile				Maximum concentration	Detection frequency
	75th	90th	95th	99th		
Sulfadiazine	–[a]	–	–	79.8	96.3	2.8
Sulfathiazole	–	–	–	315	636	3.7
Sulfapyridine	–	–	–	448	621	4.7
Sulfachlorpyridazine	–	–	–	43.6	60.6	2.8
Sulfamerazine	–	2.41	4.55	181	341	14.0
Sulfadimethoxine	–	3.68	8.77	54.9	56.6	13.1
Sulfamonomethoxine	–	–	–	144	221	3.7
Sulfamethoxazole	–	–	–	125	180	2.8
Sulfadimidine	–	–	–	120	160	1.9
Trimethoprim	–	0.141	1.92	27.8	1.27×10^3	10.3
∑SAs[b]	1.25	6.73	26.9	2.01 $\times 10^3$	2.15×10^3	30.8
Oxytetracycline	–	–	–	–	897	0.9
Tetracycline	–	–	–	31.7	332	1.9
Doxycycline	–	–	–	47.9	51.1	1.9
∑TCs	–	–	–	82.7	1.23×10^3	2.8
Enoxacin	–	–	1.11	25.7	47.1	6.5
Enrofloxacin	–	0.981	4.92	127	259	17.8
Ofloxacin	–	5.77	33.3	296	585	13.1
Norfloxacin	–	–	6.38	102	109	6.5
Ciprofloxacin	–	–	3.88	283	3.29×10^3	7.5
∑FQs	0.0314	9.88	197	742	3.58×10^3	25.2
Cefaclor	–	–	–	–	2.26×10^3	0.9
Cefoperazone	–	–	7.61	9.62	10.3	6.5
∑CFs	–	–	8.19	10.3	2.26×10^3	7.5
Florfenicol	–	–	–	180	255	2.8
Chloramphenicol	–	–	–	152	276	2.8
∑CAPs	–	–	–	331	531	2.8
Erythromycin	–	–	–	179	4.74×10^3	4.7
Azithromycin	–	–	–	90.7	672	4.7
Clarithromycin	–	–	–	40.0	65.5	4.7
Roxithromycin	–	–	–	7.70	32.7	2.8
∑MLs	–	–	12.9	317	5.51×10^3	6.5
Lincomycin	–	–	–	155	3.82×10^3	3.7
Penicillin	–	–	–	35.6	155	1.9
∑Total	7.22	61.7	531	5.15 $\times 10^3$	1.09×10^4	41.1

[a] Indicates values below the LODs, i.e., not detected.
[b] Trimethoprim was included in the calculation of ∑SAs due to their similar antibacterial mechanisms.

antibiotics are used only for animals, seven only for humans, and 16 for both animals and humans in China (Table S1); their detection frequencies in the serum were 17.8%, 23.4%, and 29.0%, respectively (Fig. S1). As a result, we speculated that the study participants were exposed to antibiotics via multiple sources such as dietary intake of contaminated foods and the common use and prescription of antibiotics.

The total antibiotic concentrations varied greatly in all serum samples, ranging from not detected to 1.09×10^4 ng/mL (Table 1, Fig. 1). Concentrations of ∑SAs, ∑TCs, ∑FQs, ∑CAPs, ∑CFs, ∑MLs, penicillin, and lincomycin in the detected serum samples ($n = 44$) ranged from 0.353 to 2.15×10^3 ng/mL, 51.1 to 1.23×10^3 ng/mL, 0.0628 to 3.58×10^3 ng/mL, 228 to 531 ng/mL, 6.96 to 2.26×10^3 ng/mL, 1.98 to 5.51×10^3 ng/mL, 37.9 to 155 ng/mL, and 42.5 to 3.82×10^3 ng/mL, respectively (Fig. 1). A notable inter-individual variability in the detected antibiotic concentrations was observed in the serum samples, which may be mainly related to the difference in the habits of drug usage among these adults. The total antibiotic concentrations in 26.2% of the serum samples (i.e., 63.6% of the detected samples) were between the LODs and 20.0 ng/mL. The serum concentrations of 5 antibiotics (trimethoprim, ciprofloxacin, cefaclor, lincomycin and erythromycin) reached above 1000 ng/mL in 3.7% of the samples, with the highest being 4.74×10^3 ng/mL for erythromycin (Table 1). Furthermore, logarithm-transformed octanol-water partition coefficient ($\log K_{OW}$) values of these five antibiotics are below 1, except for the $\log K_{OW}$ of erythromycin, which was 3.06 (Table S1); thus, they belong to low accumulation compounds in the living organisms. In particular, the $\log K_{OW}$ of cefaclor is only −0.21. In addition, it should be noted that these antibiotics have relatively low biological half-lives of <20 h, except for azithromycin, which is as high as 60 h (Table S1), suggesting their fast metabolic rates in human bodies. Therefore, their high concentrations in the human serum were most likely the result of a continuous intake of these antibiotics over the long term, which might be via daily dietary intake of contaminated foods and the common use and prescription of antibiotics. For example, the maximum concentration of lincomycin ($\log K_{OW} = 0.6$), a veterinary antibiotic (Kemper, 2008), reached 3.82×10^3 ng/mL in the serum of these adults, which indicated that it was mainly from daily dietary intake.

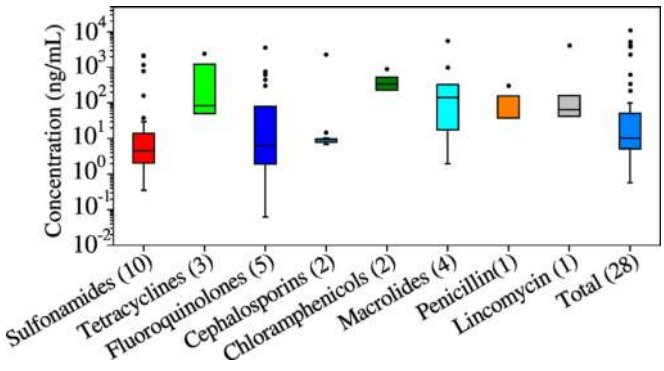


Fig. 1. Concentrations of each antibiotic category in the detected serum samples ($n = 44$). Low, middle and high bars in the box indicate 25%, 50% and 75% percentile concentrations, respectively. The data in parentheses represent the numbers of the detected antibiotics in each category.

3.2. Relations of antibiotic concentrations with gender, BMI, or age

Fig. 2 shows the geometric means of the concentrations of different antibiotic categories, classified by their antibacterial mechanisms and usages in the detected serum samples in relation to (a) gender, (b) BMI or (c) age group. The concentrations of the veterinary antibiotics in males were significantly higher ($p < 0.05$) than those in females (Fig. 2a). This significant sex-related difference could be affected by age and BMI. To exclude their possible effects, a multiple linear regression model was thus implemented. The result shows that the relation was not significant after adjusting for age and BMI (Table S5). The total antibiotic concentrations in males were also higher than those in females but not significant ($p > 0.05$). A significant sex-related difference of antibiotics in the urine of children has been previously reported (Wang et al., 2015; Wang et al., 2016a). For example, boys had a significantly higher exposure risk to sulfonamides than girls (Wang et al., 2015). One possible explanation is the different food habits, lifestyles, and morbidities of various diseases between males and females, which will affect the intake of antibiotics. The sex differences in dieting behavior indicate that men consume more calories than women, whereas eating disorders are much more prevalent in females (Rolls et al., 1991), which may unintentionally lower the antibiotic exposure risk of females via intake of various contaminated foods relative to males.

A significant difference ($p < 0.05$) in the sulfonamides between different BMI groups (BMI < 24 and BMI ≥ 24) was observed, regardless of whether adjusting for gender and age (Fig. 2b and Table S5). Higher levels of antibiotics in the normal weight group (BMI < 24) were also found for the other antibiotic categories, except for the fluoroquinolones, but the differences were not significant ($p > 0.05$). These differences suggest that antibiotic exposure can affect body weight, which may be due to the influence of different bioaccumulation profiles, exposure pathways or exposure doses. However, we failed to conduct a questionnaire survey on diet and the usage of antibiotics due to the improper or incomplete information. Additionally, more anthropometric data (e.g., percent body fat) is necessary to adequately elucidate the potential association between body weight and antibiotic use in these adults.

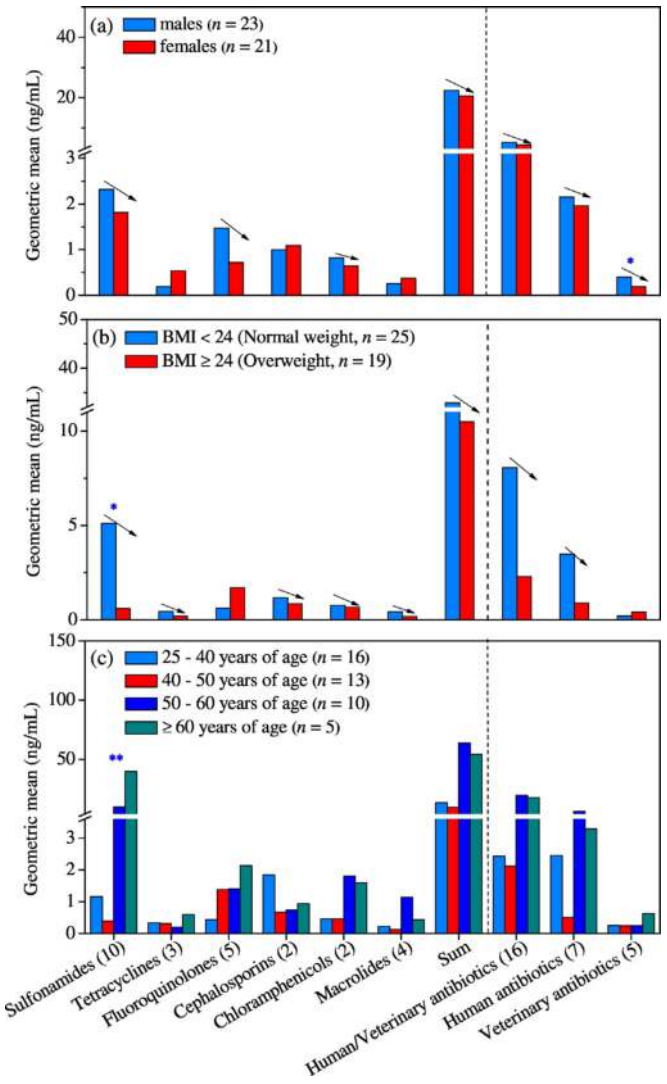


Fig. 2. Geometric means of the concentrations of antibiotic categories, classified by their antibacterial mechanisms and usages in the detected serum samples ($n = 44$) in relation to (a) gender, (b) body mass index (BMI) and (c) age group (*: 0.01 < p-value < 0.05, **: p-value < 0.01).

The concentrations of different antibiotic categories among different age groups were compared. An age categorization that defined young adults as persons 20 to 59 years of age and older adults as persons 60 years of age and older was used in the present study according to the study by Kuczmarski et al. (2001). To explore the age-related change in the antibiotic body burden, the young adults were further divided into three groups by an age range of 10, the standard deviation of the ages. Fig. 2c shows that different antibiotic categories demonstrated different concentration profiles related to age. There was a significant age-related difference ($p < 0.05$) for the sulfonamides in the serum samples (Fig. 2c and Table S5) even after adjusting for gender and BMI. Moreover, except for the fluoroquinolones, the concentration trends for the other antibiotic categories in the samples first decreased and, then, increased in the 25–60 age range. The levels of the fluoroquinolones in the serum had an increasing trend with increasing ages. Overall, the concentrations for all antibiotic categories, except for the fluoroquinolones and the tetracyclines, were the lowest in the 40–50 age group. The antibiotic levels were significantly higher ($p < 0.05$) in the adults in the ≥50 age group than those in the 25–50 age group. A recent finding in the urine of children has also reported that children in the age 10–11 group had a lower antibiotic exposure risk than those in the 8–10 age group (Wang et al., 2015). There may be two reasons for our interesting observation: i) advanced age accompanied by many physiologic alterations (e.g., lower numbers of gut microorganisms and reduced species diversity in the bodies of elderly individuals) may have negative effects on the metabolism and excretion process of antibiotics (Ljungberg and Nilssonehle, 1987; O'Toole and Claesson, 2010; Woodmansey et al., 2004); and ii) for adults, elderly individuals are more susceptible to respiratory and skin infections due to their declining immune response (Gardner, 1980), which may lead to the consequence that antibiotics are more frequently prescribed to elderly individuals than to the young people. Unfortunately, the history of recent prescribed antibiotic use by this population was not clearly understood.

The detection frequencies of antibiotics in the positive detected samples ($n = 44$) exhibited the same trends as the observed concentration profiles in relation to gender, age or BMI (Fig. S2), and no significant differences were found ($p > 0.05$). The similar sex-related difference in the frequencies of all detected antibiotics in the urine of children was reported by Wang et al. (2015). In addition, we did not analyze the relations of individual antibiotics between different groups because the detection frequencies of individual antibiotics were so low (Table 1) that the statistical results would not be persuasive despite the substitution of non-detectable values with 1/2 LODs and the exclusion of the undetected samples.

3.3. Human risks of antibiotics

Human health risks of antibiotic blood exposure for adults were assessed using the *ER* values of these antibiotics in the serum. The *ER* values of twenty detected antibiotics were calculated (Fig. 3) even though 28 antibiotics were detected because the H_TPC values of the remaining antibiotics were not available. The *ER*s for these adults ranged from 1.12×10^{-5} for cefoperazone to 1.74 for azithromycin. More than 80% of the adults were exposed to these antibiotics at the level of <0.9 μg/kg/day. More than half of the adults were exposed to these antibiotics at the level of approximately 0.5 (geometric mean: 0.6) μg/kg/day, which was much lower than that of children from Shanghai, China (Wang et al., 2016b).

For almost all of the adults investigated, no human health risks to exposure to these antibiotics were identified, even at such exposure levels in their serum. Only azithromycin had a relative high *ER* value (1.74) in one serum sample, indicating a high risk of pharmacological effects occurring. This finding might be related to its low human therapeutic plasma concentration (H_TPC: 0.04 μg/mL) and relatively high half-life ($t_{1/2}$: 60 h) in human blood (Table S1). Another explanation may be the common use and prescription of this drug.

Two limitations of this improved model based on the "read-across" hypothesis should be borne in mind. First, it might not always be the case that a chemical will exert a toxicological response only when above its pharmacological effect concentration, even though the model accounts for inter-individual variability by applying a 10-fold uncertainty factor. Second, the model only assesses human health risks in terms of causing pharmacological effects, without considering microbiome disruption, bacterial resistance or any other adverse effects. Thus, although there are no risks in terms of a therapeutically active dose, there may still be risks related to antibiotic resistance. According to pharmacodynamic models, resistant bacteria can be selected only at concentrations between the minimal inhibitory concentration (MIC) of the susceptible wild-type population (MIC_{susc}) and that of the resistant bacteria (MIC_{res}) (Drlica, 2003; Drlica and Zhao, 2007). For example, the MICs of tetracycline for the susceptible *Salmonella enterica* (Var. Typhimurium LT2) wild-type and resistant mutants (resistance marker: Tn*10d*Tet strains) are 1.5 and 128 μg/mL, respectively; the MICs of ciprofloxacin for *Escherichia coli* wild-type and one of its resistant markers (*gyrA* S83L) are 0.023 and 0.38 μg/mL, respectively (Gullberg et al., 2011). Thus, exposure to such antibiotic concentrations may result in enrichment for the resistant mutants of *E. coli* in 3% of these adults but not for *S. typhimurium*.

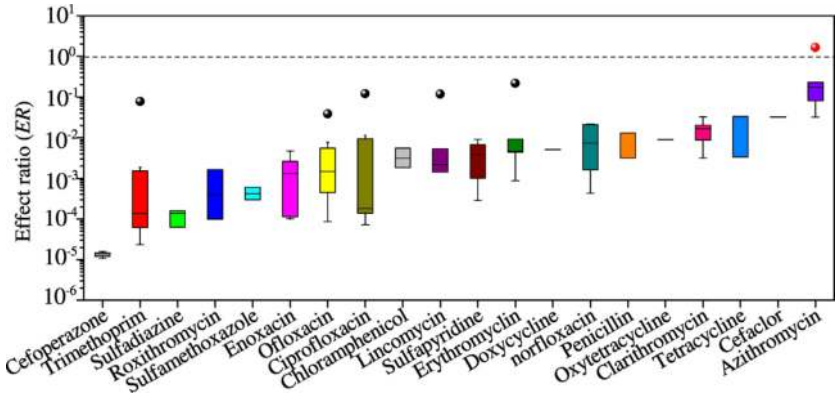


Fig. 3. Effect ratios (*ERs*) of 20 antibiotics for adults in a general population from North China. The low, middle and high bars in the box indicate 25%, 50% and 75% percentile concentrations, respectively.

4. Conclusions

Our work is the first to report the human serum concentrations of 40 antibiotics in a general population. These pilot data are very helpful for improving the understanding of the long-term effects of antibiotics on human health and for identifying susceptible populations that are at high risk to antibiotic exposure for regulators. First, the adults in the study population suffered from a multi-source and combined antibiotic exposure, and the maximum concentrations were as high as 4.74 $\times 10^3$ ng/mL for erythromycin. Moreover, concentrations of the veterinary antibiotics in males were significantly higher than those in females. Sulfonamides exhibited significant differences between the different BMI groups and age groups. However, more studies are warranted to further clarify the reasons behind the relations of antibiotics with gender, BMI, or age. Finally, there were no health risks for almost all of the adults exposed to these antibiotics although pharmacological effects may occur occasionally. Given that the risks associated with antibiotic resistance are not considered in conventional assessment models, developing new risk assessment methods for antibiotic resistance in human bodies in the future is urgently needed. In addition, some under-predicted risks such as synergistic effects will occur while humans suffer from the combined exposure to these antibiotics. However, the combined exposure risks of these antibiotics in humans also remain to be further elucidated.

Conflicts of interest

The authors have declared that no conflicts of interest exist.

Acknowledgements

Financial support by the National Basic Research Program of China (2013CB430403) and the National Natural Science Foundation of China (21677023) is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.04.216.

References

Bosca, F., 2012. Seeking to shed some light on the binding of fluoroquinolones to albumins. J. Phys. Chem. B 116 (11), 3504–3511.
Bound, J.P., Voulvoulis, N., 2004. Pharmaceuticals in the aquatic environment - a comparison of risk assessment strategies. Chemosphere 56 (11), 1143–1155.
Chen, H., Liu, S., Xu, X.R., Liu, S.S., Zhou, G.J., Sun, K.Y., Zhao, J.L., Ying, G.G., 2015. Antibiotics in typical marine aquaculture farms surrounding Hailing Island, South China: occurrence, bioaccumulation and human dietary exposure. Mar. Pollut. Bull. 90 (1–2), 181–187.
Cox, L.M., Blaser, M.J., 2015. Antibiotics in early life and obesity. Nat. Rev. Endocrinol. 11 (3), 182–190.
Drlica, K., 2003. The mutant selection window and antimicrobial resistance. J. Antimicrob. Chemother. 52, 11–17.
Drlica, K., Zhao, X.L., 2007. Mutant selection window hypothesis updated. Clin. Infect. Dis. 44, 681–688.
Gao, L.H., Shi, Y.L., Li, W.H., Liu, J.M., Cai, Y.Q., 2012. Occurrence, distribution and bioaccumulation of antibiotics in the Haihe River in China. J. Environ. Monit. 14 (4), 1248–1255.
Gardner, I.D., 1980. The effect of aging on susceptibility to infection. Rev. Infect. Dis. 2 (5), 801–810.
Gavalchin, J., Katz, S.E., 1994. The persistence of fecal-borne antibiotics in soil. J. AOAC Int. 77 (2), 481–485.
Ginsberg, G., Hattis, D., Sonawane, B., 2004. Incorporating pharmacokinetic differences between children and adults in assessing children's risks to environmental toxicants. Toxicol. Appl. Pharmacol. 198 (2), 164–183.
Gullberg, E., Cao, S., Berg, O.G., Ilbäck, C., Sandegren, L., Hughes, D., Dan, I., Andersson, D.I., 2011. Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog. 7 (7), e1002158.
Habif, T.P., 2006. Doxycycline-induced phototoxicity. N. Engl. J. Med. 355 (2), 182.
Hamscher, G., Pawelzick, H.T., Sczesny, S., Nau, H., Hartung, J., 2003. Antibiotics in dust originating from a pig-fattening farm: a new source of health hazard for farmers? Environ. Health Perspect. 111 (13), 1590–1594.
He, X.T., Wang, Z.H., Nie, X.P., Yang, Y.F., Pan, D.B., Leung, A.O.W., Cheng, Z., Yang, Y.T., Li, K.B., Chen, K.C., 2012. Residues of fluoroquinolones in marine aquaculture environment of the Pearl River Delta, South China. Environ. Geochem. Health 34 (3), 323–335.
Hektoen, H., Berge, J.A., Hormazabal, V., Yndestad, M., 1995. Persistence of antibacterial agents in marine-sediments. Aquaculture 133 (3–4), 175–184.
Huggett, D.B., Cook, J.C., Ericson, J.F., Williams, R.T., 2003. A theoretical model for utilizing mammalian pharmacology and safety data to prioritize potential impacts of human pharmaceuticals to fish. Hum. Ecol. Risk. Assess. 9 (7), 1789–1799.
Kemper, N., 2008. Veterinary antibiotics in the aquatic and terrestrial environment. Ecol. Indic. 8 (1), 1–13.
Kim, S., Eichhorn, P., Jensen, J.N., Weber, A.S., Aga, D.S., 2005. Removal of antibiotics in wastewater: effect of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process. Environ. Sci. Technol. 39 (15), 5816–5823.
Kuczmarski, M.F., Kuczmarski, R.J., Najjar, M., 2001. Effects of age on validity of self-reported height, weight, and body mass index: findings from the Third National Health and Nutrition Examination Survey, 1988–1994. American Dietetic Association]–>J. Am. Diet. Assoc. 101 (1), 28–34.
Kwok, W.H., Leung, D.K.K., Leung, G.N.W., Wan, T.S.M., Wong, C.H.F., Wong, J.K.Y., 2010. Screening of drugs in equine plasma using automated on-line solid-phase extraction coupled with liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1217 (19), 3289–3296.
Lam, H.M., Remais, J., Fung, M.C., Xu, L.Q., Sun, S.S.M., 2013. Food supply and food safety issues in China. Lancet 381 (9882), 2044–2053.
Leung, H.W., Jin, L., Wei, S., Tsui, M.M.P., Zhou, B.S., Jiao, L.P., 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, B., Zhang, T., 2013. Different removal behaviours of multiple trace antibiotics in municipal wastewater chlorination. Water Res. 47 (9), 2970–2982.
Li, Y.B., Xu, J., Wang, F., Wang, B., Liu, L.Q., Hou, W.L., Fan, H., Tong, Y.Q., Zhang, J., Lu, Z.X., 2012. Overprescribing in China, driven by financial incentives, results in very high use of antibiotics, injections, and corticosteroids. Health Aff. 31 (5), 1075–1082.
Liu, J.L., Wong, M.H., 2013. Pharmaceuticals and personal care products (PPCPs): a review on environmental contamination in China. Environ. Int. 59, 208–224.
Ljungberg, B., Nilssonehle, I., 1987. Pharmacokinetics of antimicrobial agents in the elderly. Rev. Infect. Dis. 9 (2), 250–264.
Makey, C.M., McClean, M.D., Braverman, L.E., Pearce, E.N., He, X.M., Sjödin, A., Weinberg, J.M., Webster, T.F., 2016. Polybrominated diphenyl ether exposure and thyroid function tests in North American adults. Environ. Health Perspect. 124 (4), 420–425.

Mikkelsen, K.H., Allin, K.H., Knop, F.K., 2016. Effect of antibiotics on gut microbiota, glucose metabolism and body weight regulation: a review of the literature. Diabetes Obes. Metab. 18 (5), 444–453.
Mueller, S., Saunier, K., Hanisch, C., Norin, E., Alm, L., Midtvedt, T., Cresci, A., Silvi, S., Orpianesi, C., Verdenelli, M.C., Clavel, T., Koebnick, C., Zunft, H.J.F., Dore, J., Blaut, M., 2006. Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study. Appl. Environ. Microbiol. 72 (2), 1027–1033.
Nathan, C., Cars, O., 2014. Antibiotic resistance - problems, progress, and prospects. N. Engl. J. Med. 371 (19), 1761–1763.
O'Toole, P.W., Claesson, M.J., 2010. Gut microbiota: changes throughout the lifespan from infancy to elderly. Int. Dairy J. 20 (4), 281–291.
Rand-Weaver, M., Margiotta-Casaluci, L., Patel, A., Panter, G.H., Owen, S.F., Sumpter, J.P., 2013. The read-across hypothesis and environmental risk assessment of pharmaceuticals. Environ. Sci. Technol. 47 (20), 11384–11395.
Renwick, A.G., 1998. Toxicokinetics in infants and children in relation to the ADI and TDI. Food Addit. Contam. 15, 17–35.
Riley, L.W., Raphael, E., Faerstein, E., 2013. Obesity in the United States - dysbiosis from exposure to low-dose antibiotics? Front. Public Health 1, 61–69.
Rolls, B.J., Fedoroff, I.C., Guthrie, J.F., 1991. Gender differences in eating behavior and body weight regulation. Health Psychol. 10 (2), 133–142.
Sanchez, A.R., Rogers, R.S., Sheridan, P.J., 2004. Tetracycline and other tetracycline-derivative staining of the teeth and oral cavity. Int. J. Dermatol. 43 (10), 709–715.
Thomas, G.O., Wilkinson, M., Hodson, S., Jones, K.C., 2006. Organohalogen chemicals in human blood from the United Kingdom. Environ. Pollut. 141 (1), 30–41.
Topp, E., Renaud, J., Sumarah, M., Sabourin, L., 2016. Reduced persistence of the macrolide antibiotics erythromycin, clarithromycin and azithromycin in agricultural soil following several years of exposure in the field. Sci. Total Environ. 562, 136–144.
Wang, X.H., Lin, A.Y.C., 2012. Phototransformation of cephalosporin antibiotics in an aqueous environment results in higher toxicity. Environ. Sci. Technol. 46 (22), 12417–12426.
Wang, H.X., Wang, B., Zhao, Q., Zhao, Y.P., Fu, C.W., Feng, X., Wang, N., Su, M.F., Tang, C.X., Jiang, F., Zhou, Y., Chen, Y., Jiang, Q.W., 2015. Antibiotic body burden of Chinese school children: a multisite biomonitoring-based study. Environ. Sci. Technol. 49 (8), 5070–5079.
Wang, H.X., Wang, N., Wang, B., Fang, H., Fu, C.W., Tang, C.X., Jiang, F., Zhou, Y., He, G.S., Zhao, Q., Chen, Y., Jiang, Q.W., 2016a. Antibiotics detected in urines and adipogenesis in school children. Environ. Int. 89-90, 204–211.
Wang, H.X., Wang, N., Wang, B., Zhao, Q., Fang, H., Fu, C.W., Tang, C.X., Jiang, F., Zhou, Y., Chen, Y., Jiang, Q.W., 2016b. Antibiotics in drinking water in Shanghai and their contribution to antibiotic exposure of school children. Environ. Sci. Technol. 50 (5), 2692–2699.
Wawruch, M., Bozekova, L., Krcmery, S., Kriska, M., 2002. Risks of antibiotic treatment. Bratisl. Lek. Listy 103 (7–8), 270–275.
WHO, 2014. Antimicrobial Resistance: Global Report on Surveillance.
Woodmansey, E.J., McMurdo, M.E.T., Macfarlane, G.T., Macfarlane, S., 2004. Comparison of compositions and metabolic activities of fecal microbiotas in young adults and in antibiotic-treated and non-antibiotic-treated elderly subjects. Appl. Environ. Microbiol. 70 (10), 6113–6122.
Yamaguchi, T., Okihashi, M., Harada, K., Konishi, Y., Uchida, K., Do, M.H.N., Bui, H.D.T., Nguyen, T.D., Do Nguyen, P., Van Chau, V., Van Dao, K.T., Nguyen, H.T.N., Kajimura, K., Kumeda, Y., Bui, C.T., Vien, M.Q., Le, N.H., Hirata, K., Yamamoto, Y., 2015. Antibiotic residue monitoring results for pork, chicken, and beef samples in Vietnam in 2012–2013. J. Agric. Food Chem. 63 (21), 5141–5145.
Zhi, C.P., Lv, L.C., Yu, L.F., Doi, Y.H., Liu, J.H., 2016. Dissemination of the mcr-1 colistin resistance gene. Lancet Infect. Dis. 16 (3), 292–293.
Zhu, Y.G., Johnson, T.A., Su, J.Q., Qiao, M., Guo, G.X., Stedtfeld, R.D., Hashsham, S.A., Tiedje, J.M., 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proc. Natl. Acad. Sci. U. S. A. 110 (9), 3435–3440.