Environment International 108 (2017) 246–252

Contents lists available at ScienceDirect

Environment International

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

Veterinary antibiotics in food, drinking water, and the urine of preschool children in Hong Kong

Na Li[a], Keith W.K. Ho[a], Guang-Guo Ying[b,*], Wen-Jing Deng[a,*]

[a] Department of Science and Environmental Studies, The Education University of Hong Kong, Tai Po, N.T., Hong Kong Special Administrative Region
[b] The Environmental Research Institute, MOE Key Laboratory of Environmental Theoretical Chemistry, South China Normal University, Guangzhou 510006, China

ARTICLE INFO

Keywords:
Veterinary antibiotics
Preschool children
Raw and cooked food
Urinalysis

ABSTRACT

Due to the harmful effects of veterinary antibiotics (VAs) residues in food on children's health, urine samples from 31 preschool and primary school children were analyzed for 13 common VAs. Samples of raw and cooked pork, chicken, fish, milk and drinking water from the children's living areas were also analyzed for residual VAs. Urinalysis revealed one to four target antibiotics in 77.4% of the sample group, with concentrations as high as 0.36 ng/mL. Norfloxacin and penicillin had the highest detection rates (48.4% and 35.5%, respectively), with median concentrations of 0.037 and 0.13 ng/mL, respectively. The VA burden of children in HK was lower than that in Shanghai. Enrofloxacin, penicillin, and erythromycin were the most detected VAs in raw and cooked food. Only oxytetracycline was detected in terminal tap water, and none were detected in milk. Tetracycline and doxycycline hyclate were detected in organic eggs (up to 7.1 ng/g) and regular eggs (up to 6.6 ng/g), which were common in children's diets. Traditional Chinese cooking processes did not completely eliminate VAs, and the concentrations of some VAs increased, especially after frying and roasting. The estimated daily intake (EDI) results show that the contribution of dietary intake and that based on the urine concentrations of VAs were far below the acceptable daily intake (ADI). The EDIs from urine were significantly lower than those based on cooked foods. The highest level of achievement percentage (LAP) based on dietary consumption and urine concentrations were 39.7% and 1.79%, respectively, and thus current levels of exposure to VAs would not seem to pose a risk to children's health. However, harmful effects of residual VAs during developmental periods may occur with exposure to much lower doses than those considered harmful to adults, and further investigation of these emerging pollutants is urgently encouraged.

1. Introduction

Antibiotics have been widely used to treat infectious diseases in humans and to prevent infectious diseases in animals. They are also frequently used as growth promoters in aquaculture and agriculture (Kümmerer, 2009). Driven by the huge demand for livestock and marine products, the rate of antibiotic use has increased rapidly (Cabello, 2006; Zhang et al., 2012). Van Boeckel et al. (2015) estimated that the total global use of antibiotics in livestock was 63,151 tons in 2010 and would rise by 67% by 2030, nearly doubling in China, South Africa, Brazil, India, and Russia. As a result of this extensive antibiotic use, a variety of antibiotic residues have been found in meat, milk, and egg products (Leung et al., 2013; Liu and Wong, 2013).

The health effects of antibiotic residues in food have attracted increasing concern in recent years. Contamination with antibiotics has been found to generate various harmful effects in the human body at low concentrations (Bouki et al., 2013; Gao et al., 2012). It has been reported that exposure to specific antibiotics is related to the development of obesity (Ajslev et al., 2011; Bailey et al., 2014; Thuny et al., 2010) and type 2 diabetes with glucose homeostasis disturbances (Chou et al., 2013). Antibiotics can interfere with the human gut microbiota and cause marked alterations in some individuals (Dethlefsen and Relman, 2011; Jakobsson et al., 2010; Jernberg et al., 2007). In addition, even low concentrations of antibiotics can cause multidrug resistance (Kohanski et al., 2010) and increase the prevalence of antibiotic resistance genes (Wright, 2007). Even short-term antibiotic administration can allow resistant bacterial populations to stabilize and persist in the human body for years (Jakobsson et al., 2010).

Children are more vulnerable than adults to antibiotics (Bailey et al., 2014; Schug et al., 2011). Experiments in mice found that antibiotic exposure early in life with low concentrations had effects on metabolism, gut microbiota, and adipogenesis, which could lead to obesity and diabetes (Cho et al., 2012; Cox and Blaser, 2015; Cox et al., 2014). Epidemiological studies have also observed that early-life

---

* Corresponding authors.
E-mail addresses: guangguo.ying@m.scnu.edu.cn (G.-G. Ying), wdeng@eduhk.hk (W.-J. Deng).

http://dx.doi.org/10.1016/j.envint.2017.08.014
Received 19 April 2017; Received in revised form 5 July 2017; Accepted 22 August 2017

exposure to antibiotics was positively related to the risk of childhood obesity (Azad et al., 2014; Bailey et al., 2014; Murphy et al., 2014; Trasande et al., 2013). These harmful effects of antibiotics during developmental periods may occur with exposure to much lower doses than those considered harmful to adults. Health effects early in life are important, because adult health and disease might have their origins in the prenatal and early postnatal environment (Hanson and Gluckman, 2011).

In reality, children are always exposed to the risk of antibiotics because there are multiple antibiotic exposure pathways, and long-term, low-dose exposure through contaminated food may be important for child health (Cai et al., 2008; Li et al., 2012; Tao et al., 2012; Yiruhan et al., 2010). Many studies have revealed that children are widely exposed to antibiotics from dietary intake at low dosage levels (Ji et al., 2010a,b; Wang et al., 2015). A recent statistical analysis revealed adipogenesis and obesity in school children in Shanghai who were exposed to some types of veterinary antibiotics (VAs), which came mainly from food and drinking water (Wang et al., 2016a,b). Another survey of > 1000 children in eastern China found a heavy antibiotic body burden in an analysis of 18 antibiotics in their urine (Wang et al., 2015). However, current epidemiological investigation inevitably ignore VA exposure from diary intake, and few studies have determined the VA residues in cooked food (Azad et al., 2014; Murphy et al., 2014; Trasande et al., 2013).

There is an urgent need to estimate the many kinds of antibiotic residues in food, especially in meat, milk, and eggs, which are heavily consumed by Hong Kong's population. Hong Kong has the densest population in the world. The daily meat consumption of the > 7 million people in Hong Kong was 4573 pigs and 23 tons of poultry in 2015, and the total fish pond production in Hong Kong amounted to 2543 tons (AFCD, 2017). China and the United States, which are the top two countries in global antibiotic consumption (46% of the total) in food animal production, are two important meat suppliers for Hong Kong. The results of our previous study and recent monitoring studies suggest that antibiotics are widespread in Hong Kong and the Pearl River estuary (Deng et al., 2016; Huang et al., 2012; Peng et al., 2012; Richardson et al., 2005; Xu et al., 2007; Yang et al., 2011). Because of Hong Kong's high meat intake and wide range of pollution, VAs might pose risks to humans (http://www.cfs.gov.hk/cindex.html). However, previous studies in Hong Kong have mostly investigated the occurrence and fate of antibiotics in the aquatic environment or sewage. Data regarding antibiotic intake are limited. The current risks associated with antibiotics may be substantially underestimated.

To learn more about VAs uptake from food, preschool children's VAs burden from dairy food was studied on a small scale in Hong Kong in this study. As the human body metabolizes antibiotics and a great proportion is excreted in urine, the urinary concentration of antibiotics is a potential exposure biomarker to assess children's internal exposure to antibiotics. Children 4 to 6 years of age (no antibiotic medicine had been taken in recent month) were selected, and their urinary concentrations of antibiotics were measured with high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry (HPLC-ES-MS/MS). Five classes of VAs commonly used in animal industry, including fluoroquinolones, tetracyclines, sulfonamides, beta-lactams, and macrocyclics, were selected as the target chemicals. The influence of cooking procedures which could change the content of VAs in food (Furusawa and Hanabusa, 2002; Heshmati, 2015) and the acceptable daily intake (ADI) were also considered in this survey.

2. Materials and methods

2.1. Chemicals

Antibiotics (> 98% purity) of internal standard were used, including three tetracyclines (tetracycline [TC], doxycycline hyclate [DTC], and oxytetracycline [OTC]), three sulfonamides (sulfadimethoxine [SDM], sulfamerazine [SMR], and sulfamethoxazole [SMX]), four fluoroquinolones (ofloxacin [OFC], enrofloxacin [EFC], ciprofloxacin [CIP], and norfloxacin [NFC]), two macrolides (lincomycin hydrochloride [LIN] and erythromycin [ETM]), and one beta-lactam (penicillin [PEN]) were obtained from Sigma-Aldrich (USA). Formic acid (99%) was obtained from Fluka (USA). Disodium ethylenediaminetetraacetic acid, NH4Ac, NaH2PO4·2H2O, and phosphoric acid were purchased from Sinopharm (China). HPLC-grade acetonitrile and methanol solvents were purchased from Fisher Scientific (USA). Standard solutions were diluted to 500 μg/mL with methanol and stored at − 20 °C. Working solutions were prepared before analysis.

2.2. Food, drinking water, and urine collection

Urine samples were collected by the participants upon awakening, immediately transported to the laboratory in an ice chest, and frozen at − 20 °C for analysis. The samples of raw food (pork, chicken, egg, fish; 50 g each) were purchased from different markets close to the children's residential area and from different suppliers. Half of the raw food were cooked immediately by some traditional Chinese cooking processes: boiling (ingredients were immersed into water, produced at 100 °C for about 20–40 min); stir frying (ingredients were fried in a small amount of very hot oil while being stirred in a wok, and then added with sauce, produced at 100–200 °C for about 5–20 min); steaming (ingredients were cooked using steam, produced at 100 °C for about 30 min); roasting (ingredients were cooked using the oven and dry-heat, produced at 150–200 °C for about 5–20 min); and braising (ingredients were cooked using a small amount of water and the food was cooked over low heat for a longer time, produced at 100 °C for about 30–60 min). The raw and cooked foods were all collected. Milk samples were from the brand consumed daily by the children. Drinking water samples were collected from residential tap water in Tai Po, Hong Kong. The water samples were boiled in a kettle and then cooled naturally. Two control samples of organic chicken and egg (labeled without any antibiotics) were collected at the same time. After collection, the samples were stored in an ice box (− 4 °C), transported to the laboratory, and stored at − 20 °C until detection.

2.3. Antibiotic analysis of food, drinking water, and urine samples

Thirteen common antibiotics were selected from five categories, including three tetracyclines (TC, DTC, OTC), three sulfonamides (SDM, SMR, SMX), four fluoroquinolones (OFC, EFC, CIP, NFC), two macrolides (LIN, ETM), and one beta-lactam (PEN). All samples were homogenized and stored at − 20 °C until analysis. Each 2-g tissue sample was placed in a 50-mL centrifuge tube with 200 μL of 0.1-M ethylenediaminetetraacetic acid and shaken at 2000 rpm for 2 min with a vortex mixer (Vortex Genie, Fisher Scientific). Water (1000 μL) was added to each sample, and they were re-shaken for a further 5 min and allowed to stand in the dark at 4 °C for 30 min. Acetonitrile (10 mL; HPLC grade; Honil Limited, London, UK) was added to each sample, followed with a further 10 min shake and centrifugation at 15000 rpm for 10 min. The supernatants were collected in 15-mL tubes, evaporated to dryness under a stream of nitrogen, and reconstituted in 1 mL of water. Each sample were then sonicated for 10 min and centrifuged at 16000 rpm for 10 min. The resulting supernatants were filtered with a 0.45-μm filter, and the injection volume to the liquid chromatography system was 20 μL. An internal standard method was used for quantitative analysis, and the final solution for analysis contained 1 μg/mL of the internal standard.

For the milk, drinking water, and urine samples, 1.0-mL samples were added to 200 μL of 1.0-M ammonium acetate buffer and 15 μL of β-glucuronidase aqueous solution (≥ 100,000 units/mL) from *Helix pomatia*. The mixtures were incubated in a water bath at 37 °C and extracted by solid phase. The antibiotics were eluted by 2 mL of pure methanol containing 0.2% formic acid and concentrated to dryness