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Antibiotics in the agricultural soils from the Yangtze River Delta, China

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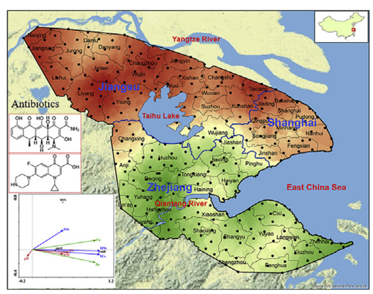
HIGHLIGHTS

- Antibiotic residuals were widely detected in agricultural soils from the YRD region of China.
- Higher concentrations of antibiotics were found in Jiangsu and Shanghai.
- Manure application and wastewater irrigation were the main sources of antibiotic inputs.
- The ARGs *tet*A, *sul*I, and *qnr*S were detected in the agricultural soils.

GRAPHICAL ABSTRACT



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ABSTRACT

This study focused on the occurrence and spatial distribution of 13 common antibiotics in the agricultural soils of the Yangtze River Delta (YRD), China. Antibiotics were detected in all the 241 soil samples (*i.e.*, 100% detection rate) with the total concentrations ranging from 4.55 to 2,010 ng/g dry weight. The concentrations of three antibiotic classes decreased in the order: quinolones (mean 48.8 ng/g) > tetracyclines (mean 34.9 ng/g) > sulfonamides (mean 2.35 ng/g). Ciprofloxacin was the prevalent compound with a mean concentration of 27.7 ng/g, followed by oxytetracycline (mean of 18.9 ng/g). A distinct spatial distribution was observed, where high concentrations of antibiotics were detected in the sites adjacent to the livestock and poultry farms. The potential sources of antibiotics in the agricultural soils were the application of manure and wastewater irrigation in this region. Risk assessment for single antibiotic compound indicated that tetracyclines and quinolones could pose a potential risk, in which doxycycline and ciprofloxacin had the most severe ecological effect in the agricultural soils. Antibiotic resistance genes (ARGs), such as *tet*A, *sul*I, and *qnr*S, were detected in 15 analyzed soil samples, and *sul*I showed significant correlations with quinolones, tetracyclines, copper, and zinc. Further studies on the distribution of other ARGs in agricultural soil at a region-scale are needed for the risk management of extensively used antibiotics and major ARGs.

1. Introduction

Antibiotics have become the most popular antimicrobial drug for human disease control and livestock growth promotion for a long time. China is one of the largest producers and consumers of antibiotics in the world. The total usage of antibiotics was 92,700 tons in 2013, and an estimated 54,000 tons of the antibiotics was discharged after human and animals use (Zhang et al., 2015). A large number of these antibiotics cannot be metabolized *in vivo*, 30–90% of which will be excreted into the environment (Hu et al., 2010; Zhao

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et al., 2010). Due to the use of organic manure/sludge and irrigation of wastewater or reclaimed water in the agricultural land, a significant amount of antibiotics, such as tetracyclines and quinolones, are found in soils (Wang et al., 2014a, 2014b; Hou et al., 2015).

Many antibiotics are biologically active in the environment (Zhou et al., 2006, 2013; Xu et al., 2013), which may pose a pressure on antibiotic resistance genes (ARGs) and bacterial communities (Ding and He, 2010; Tello et al., 2012; Xiong et al., 2015). The occurrence of ARGs in sediments, soil, and water has been observed in different countries (Pruden et al., 2006; Knapp et al., 2010; Chen et al., 2016a), which may be enriched by antibiotic residues (Cheng et al., 2016; Yang et al., 2017) and transferred into different environmental media (Chen et al., 2016a,b; Xie et al., 2016). Therefore, antibiotic may cause significant impacts on the environment and human (Tello et al., 2012).

It has been reported that soil is an important reservoir for antibiotics. For example, the levels of several antibiotics in soils in farmland around feedlots in Shanghai were 3.27–33.4 μg/g (Hu et al., 2010). The detected concentrations of antibiotics in organic farms ranged from 0.1 to 2,683 ng/g in soils (Xiang et al., 2016). Antibiotics with high concentration were detected in vegetable farmlands adjacent to livestock farms, and the detected concentration of quinolones can reach up to 1,540 ng/g (Li et al., 2011). This indicated that livestock manure is an important source of antibiotic residues in soil. After the application of manure/sludge contaminated with antibiotics to agricultural soil, the residues of antibiotics in soil can be accumulated by vegetables that are consumed by human (Hu et al., 2010).

The Yangtze River Delta (YRD) is a developed and populated region in China. The YRD region include Shanghai Municipality, Zhejiang province and Jiangsu province. As a result, the increasing need for agricultural produce from urban population led to booming agriculture in the region (Sun et al., 2016b). Our previous studies showed that organochlorine pesticides, phthalate esters, polybrominated diphenyl ethers (Sun et al., 2016b), and polychlorinated biphenyls (Sun et al., 2016a) were widely detected, which may pose ecological risks in the YRD region. Yet, little information is known on the distribution of commonly-used antibiotics in agricultural soils of the YRD region. It is important to evaluate the regional-scale distribution and the potential ecological risks of antibiotics to facilitate sustainable economic development for this rapidly developing region.

This study characterizes the concentrations and distribution patterns of tetracycline, sulfonamide and quinolone residues in agricultural soils from the YRD region of China and their potential ecological risks, as well as typical ARGs in a number of soil samples. The regional-scale evaluation of multiple antibiotics pollution in agricultural soils will provide useful information for the management of antibiotics contamination in China.

2. Materials and methods

2.1. Chemicals and reagents

The targeted antibiotics in this study were chosen according to their application in livestock industry in China. There are three classes of antibiotics, i.e., tetracyclines (TCs), quinolones (QNs) and sulfonamides (SAs). Three TCs included tetracycline (TC), doxycycline (DC), and oxytetracycline (OTC). Four QNs included ofloxacin (OFL), ciprofloxacin (CIP), norfloxacin (NOR), and enrofloxacin (ENR). Six SAs included sulfadimethoxine (SDM), sulfamethazine (SMZ), sulfamethizole (SMTZ), sulfamerazine (SMR), sulfamethoxazole (SMX), and sulfadiazine (SDZ). Simeton was used as the internal standard. The standards were obtained from J & K Chemical Ltd., USA. Acetonitrile and methanol (HPLC grade) were obtained from Sigma-Aldrich. Oasis HLB Cartridge used for purification were purchased from Waters. Deionized water (18.2 MΩ) from a Milli-Q system was used.

2.2. Sample collection

The YRD region is located in east China. In June 2014, 241 agricultural soils were collected in this region. The studied region covered approximately 45,800 km^2. The detailed information about sample collection were presented in our previous report (Sun et al., 2016b). The types of land-use of the sampling sites included paddy fields, vegetable fields, forests, uncultivated lands, and other agricultural uses.

2.3. Sample extraction and clean-up

The extraction and cleanup procedures for antibiotics were conducted according to the methods reported previously (Uslu. et al., 2007), with small modifications. Briefly, all the soil samples were purified using solid phase extraction (SPE) method after freeze drying, homogenizing and sieving through a 75 mesh sieve.

Two grams of soil were extracted by using acetonitrile (3 mL) and potassium phosphate buffer (3 mL) (pH = 4) containing 0.2 g of EDTA in a 15-mL polypropylene centrifuge tube. After being vortexed for 1 min, the sample was treated ultrasonically for 20 min, followed by centrifugation at 2600 rpm for 10 min. Afterwards, the supernatants were moved carefully to a new tube. This extraction procedure was repeated three more times with additions of 5, 5 and 4 mL of reagents. The extracts were combined and transferred to new glass bottles. Then, these extracts were concentrated with a rotary evaporator, and diluted to 500 mL to lower the concentration of organic solvents to below 5%.

The Oasis HLB Cartridge was used for the purification of each soil sample. The cartridges were preconditioned sequentially with 6 mL of methanol and 6 mL of deionized water. Then, the dilute supernatants were loaded on the cartridges at a rate of about 5 mL/min. The cartridges were then vacuum-dried after being rinsed with 12 mL of deionized water. The elution on the cartridges were performed with 0.1% acidified methanol (9 mL) at a rate of 1.0 mL/min. The analytes were then concentrated to near dryness under a gentle flow of nitrogen gas. Simeton was added and methanol was used to dissolve the final extract to 1 mL. The final extract was filtered through syringe filters (0.22 μm) before further instrumental analysis.

2.4. Instrumental analysis

The determination of antibiotics in this study was performed by using a liquid chromatography–tandem mass spectrometry system (Agilent 1260–6460), according to a previous study (Wu et al., 2014) with some modifications. A C18 column (5 μm, 2.1 × 150 mm) was used for the chromatographic separation. The mobile phase was methanol–acetonitrile (1:1, v/v) and 0.3% formic acid/water (containing 0.1% ammonium formate, v/v). The column temperature was 40 °C, and the injection volume was 20 μL. The detection was conducted with ESI source in the mode of multiple-reaction monitoring (MRM). The detailed information on instrumental parameters is listed in Supporting Information (Table S1).

2.5. DNA extraction and real-time qPCR

The current study analyzed three common ARGs (*tet*A, *sul*I, and *qnr*S) in the selected 15 sampling sites from the northeast of the YRD region, where high levels of antibiotics were found. The methods of DNA extraction and real-time qPCR were adopted from the previous literature (Chen and Zhang, 2013). Briefly, DNA