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Fluoroquinolones and β-lactam antibiotics and antibiotic resistance genes in autumn leachates of seven major municipal solid waste landfills in China

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ABSTRACT

Landfills are reservoirs of antibiotics, heavy metals, disinfectants and other emerging contaminants, and they are closely associated with the increasing prevalence of antibiotic-resistance genes (ARGs). In this study, two classes of clinical use antibiotics, i.e., fluoroquinolones (FQs) and β-lactams (BLs), twelve subtypes of their parallel ARGs, and five mobile genetic elements (MGEs), were measured in municipal solid waste (MSW) landfill leachates from seven mega-cities in China. The highest concentration of FQs was detected in Shanghai (48,326.67 ng/L), and the highest concentration of BLs was detected in Hangzhou (1304 ng/L). In landfill leachates in Suzhou, the total contents of targeted ARGs subtypes ($(1.44 \pm 4.64) \times 10^{-4}$ (ARGs/16S)) and MGEs ($7.88 \times 10^{-2} \pm 1.18 \times 10^{-1}$ (ARGs/16S)) were the highest. The relative abundance of ARGs and MGEs was significantly correlated with the contents of As and Cr, and the presence of MGEs was highly correlated with the content of Cd ($r = -0.438$, $p = 0.475$). Linear regression analysis showed that MGEs are closely associated with the abundance of genes resistant to FQs and BLs. These results suggest that the occurrences of FQs and BLs ARGs in the landfills of China are substantially influenced by heavy metals and MGEs. Regional differences concerning the antibiotics and ARGs contents in leachates were observed across seven mega-cities, and FQs were significantly correlated with the local population level ($p < 0.01$). Further, the nitrogen input to the landfills contributes significantly to the elevated levels of target ARGs.

1. Introduction

Sanitary landfilling has been a worldwide municipal solid waste (MSW) disposal practice (Eggen et al., 2010; Threedeach et al., 2012). The total amount of MSW sent to landfills has reportedly reached 350 million tons per year (Hassan and Xie, 2014). However, due to poor management, especially in developing countries, > 90% of all waste is landfilled without proper pretreatment (Zhang et al., 2010). China is the world's largest producer and consumer of antibiotics, and the consumption of antibiotics has been estimated to be ~54,000 tons per year (Zhang et al., 2015). Up to 70% of all antibiotics are released into the environment in their original form (Kümmerer, 2003) because they are poorly absorbed in humans and animals (Sarmah et al., 2006). Given the considerable input of unsorted and untreated wastes (Yu et al., 2016), a MSW landfill is commonly considered a site that has been contaminated with antibiotics (Musson and Townsend, 2009) and other antimicrobial agents containing clinical wastes (Eggen et al., 2010).

Previous investigations have shown that the unbalanced economic development influences the consumption rates of antibiotics: their usage is stable or on a moderately decreasing trend in developed countries but is drastically increased in developing countries (Van Boeckel et al., 2014). Further, in low- and middle-income countries, antibiotics use is poorly regulated. In India, the consumption of antibiotics increased by 37% between 2005 and 2010, and non-prescription

Abbreviations: FQs, fluoroquinolones; BLs, β-lactams; OFL, ofloxacin; NOR, norfloxacin; ENR, enrofloxacin; PEF, pefloxacin; CEF, cephalosporin; AMOX, amoxicillin; ARGs, antibiotic resistance genes; MSW, municipal solid waste; AMR, antimicrobial resistance; TN, total nitrogen; TP, total phosphorus; NH_4^+-N, ammonium-nitrogen; NO_3-N, nitrate-nitrogen; NO_2-N, nitrite-nitrogen; ANOVA, analysis of variation; PCA, principal components analysis; RDA, redundancy analysis; MGEs, mobile genetic elements; HGT, horizontal gene transfer; HT-qPCR, high-throughput quantitative PCR

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use accounted for ~20% of the total antibiotics use in the clinic (Laxminarayan and Heymann, 2012). The non-prescription use was estimated to be 36% in China (Sweidan et al., 2005). Thus, the clinical antibiotic usage in China reached 150 daily doses per 1000 inhabitants per day (DID), which was the highest value worldwide (Zhang et al., 2015). Although the Chinese Ministry of Health (MOH) has taken measures to curtail the overuse of antibiotics since 2012 (http://www.moh.gov.cn), the misuse of clinical antibiotics as veterinary drugs and the management of animal wastes in husbandry industries have not been systematically regulated (Larson, 2015).

A recent survey showed that β-lactams (BLs) and fluoroquinolones (FQs) are among the top three most consumed antibiotics worldwide in terms of amount (Rice, 2012). In 2013, China consumed 27,300 and 34,100 tons of FQs and BLs, respectively (Zhang et al., 2015). Among these antibiotics, the consumption of clinically important cephalosporins and fluoroquinolones has been recorded, with the highest increases among all antibiotics over the last decade, reaching 8.1×10^9 and 3.0×10^9 standard units, respectively (Van Boeckel et al., 2014). Therefore, the disposal of BLs- and FQs-containing wastes into landfills could potentially trigger the proliferation of antibiotic resistance genes (ARGs) and antibiotic-resistant pathogens (Su et al., 2017a, 2017b; Wu et al., 2015). Recent studies have shown that the release of BLs (e.g., carbapenem)-related resistance genes and faeces-born commensal pathogens from human wastes sewers (Lamba et al., 2017) and landfills (Wu et al., 2017b) into living environments has an increasing trend. For landfills, the dissemination of ARGs is highly facilitated by horizontal gene transfer (HGT) (Sun et al., 2016; Xie et al., 2014). This process is reportedly mediated by mobile genetic elements (MGEs) (Marshall and Levy, 2011), regardless of the persistent presence of antibiotics or other resistance selective agents (e.g., metals, disinfectants, detergents) (Ji et al., 2012). However, there is still a knowledge gap concerning the release or distribution of BLs and FQs and their parallel ARGs from major landfills in China, which is potentially hazardous to human health, especially in densely populated mega-cities.

Environmental factors, such as heavy metals, organic matter and toxic contents, which are commonly enriched in landfills, could also exert selective pressures on antibiotic resistance (Song et al., 2016; Su et al., 2017a, 2017b; Wang et al., 2015) and affect the dissemination of these compounds (Seiler and Berendonk, 2012). MSW landfills might be perfect antimicrobial resistance (AMR) hotspots because of this phenomenon.

In this study, four types of FQs (ofloxacin, norfloxacin, enrofloxacin, and pefloxacin) and two types of BLs (cephalosporins and amoxicillin) were measured in leachates from seven MSW landfills located near seven mega-cities in China. These landfills receive 6500 tons of MSW per day from those seven mega-cities, which are located in different regions across China and have different social-economic development levels. In addition, the levels of heavy metals, carbons and nutrients in leachates were monitored to identify how landfill conditions impact the abundance and HGT potential of ARGs in landfill leachates. By investigating the distribution of clinically important antibiotic residues and ARGs in landfills located in different socio-economic development regions, this study aims to reveal the occurrences of antimicrobial resistance risks on a national scale, and thereby develop customized strategies of ARG control in different MSW landfills.

2. Materials and methods

2.1. Sampling sites and pretreatment

Landfill leachates were collected from seven different landfills from six provinces. The selection of landfill sites was based on the geographical location, population size and economic development level. The landfill sites were Xi'an (SX-XA) Qicungou Landfill (operated since 1994, 5500 t/d), located in northwest China; Guiyang (GZ-GY), Gaoyan Landfill (operated since 2001, 2200 t/d), located in southwest China; Nanjing (JS-NJ), Shuige Landfill (operated since 1993, 3000 t/d), Suzhou (JS-SuZ) Qizishan Landfill (operated since 1993, 2500 t/d) and Shanghai (SH), Laogang Landfill (operated since 1985, 10,000 t/d), Hangzhou (ZJ-HZ) Tianzilin Landfill (operated from 1991, 6000 t/d), located in of east China; Shenzhen (GD-ShZ) Xiaping Landfill (operated since 1997, 4500 t/d), located in south China. These landfills were all located in megacities of China (> 500 million people), and Southern China is generally better developed than Northern China is. The population and economic parameters of these regions are summarized in **Table S1**. To minimize the rainfall and temperature effect on the composition of leachates, the samples were collected in three consecutive days without precipitation from September to November, autumn in China (except for Guiyang). All leachates were sampled in triplicate (2 L) from each target landfill reservoir where the fresh leachates from currently operational zones were stored before treatment. The sampling bottles were stored in iceboxes. After returning to the laboratory, leachate samples were centrifuged at 1000*g* for 8 min, and the pellets were stored at −40 °C. The supernatant was filtered through a 0.45-μm membrane. The membranes and filtrates were stored at −40 °C until further analysis.

2.2. Measurements of antibiotics

Six antibiotics belonging to two classes were selected in this study (Table 1). The target FQs included ofloxacin (OFL), norfloxacin (NOR), enrofloxacin (ENR), and pefloxacin (PEF), and the target BLs included cephalosporin (CEF) and amoxicillin (AMOX). A 50-mL aliquot of pretreated leachate was used to measure the antibiotics. The analytical method was developed by optimizing the previous isotope internal standard method (Huang et al., 2013). Prior to the analyses, the antibiotics in all samples were extracted through solid phase extraction (SPE) using tandem cartridges, including in the clean-up (CNWBOND, strong anion exchanger (SAX); 6 mL/200 mg) and extraction (Waters Oasis, Hydrophile-Lipophile Balance Number (HLB); 6 mL/500 mg)

Table 1
Target antibiotics, recovery rate and LOD (ng/L), LOQ (ng/L) in this study.

Compound	Abbreviation	CAS·NO	Scale	Purity	corporation	Recovery rate in leachates (%): Mean ± sd			LOQ	LOD
						20 ng	50 ng	100 ng		
Norfloxacin	NOR	70458-96-7	Pure product 0.1 g	99.50%	Dr.	114.0 ± 2.5	102.4 ± 1.0	80.0 ± 0.5	0.456	0.137
Ofloxacin	OFL	82419-36-1	Pure product 0.2 g	99.50%	Dr.	93.8 ± 2.8	106.9 ± 6.8	97.8 ± 3.4	0.125	0.037
Enrofloxacin	ENR	93106-60-6	Pure product 0.1 g	99.60%	Dr.	101.7 ± 12.7	106.7 ± 5.1	113.9 ± 2.5	0.334	0.1
Pefloxacin	PEF	70458-92-3	Pure product 0.1 g	71.30%	Dr.	130.3 ± 4.7	115.5 ± 1.9	107.2 ± 1.0	0.238	0.071
Norfloxacin-d5	NOR-D5	/	10 μg	99.30%	Witega					
Amoxicillin	AMOX	61336-70-7	Pure product 0.25 g	98%	Dr.	101.7 ± 11.3	122.5 ± 4.5	115.8 ± 2.3	4.289	1.287
Cephalexin	CEP	23325-78-2	Pure product 0.25 g	99%	Dr.	100.4 ± 0.8	65.2 ± 0.3	52.8 ± 0.2	0.29	0.087
Cefapirin-d4	CFP-D4	/	1 μg	/	TRC					

CAS = chemical abstract service number, LOQ = the limit of quantification, LOD = the limit of detection.