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Sulfamethoxazole, tetracycline and oxytetracycline and related antibiotic resistance genes in a large-scale landfill, China

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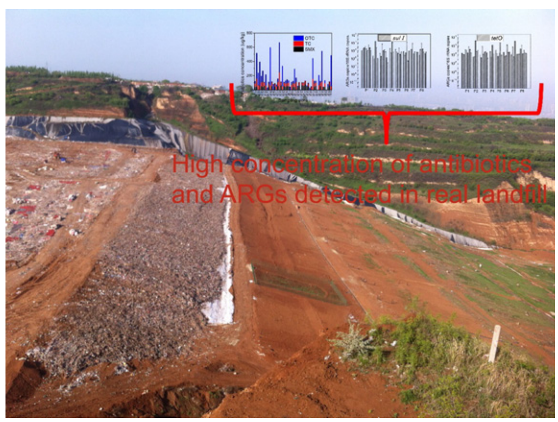
HIGHLIGHTS

- Simultaneous measure of antibiotics and ARGs at a large-scale landfill
- 3 antibiotics and 2 ARGs were detected in most locations and depths.
- Oxytetracycline were most prevalent antibiotic.
- ARGs declined with age of refuse suggesting attenuation.
- High concentrations of oxytetracycline correlated with *tetO*.

GRAPHICAL ABSTRACT



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ABSTRACT

Landfills are likely to be important reservoirs of antibiotics and antibiotic resistant genes (ARGs) as they receive unused and unwanted antibiotics and ARGs in municipal solid waste (MSW). The distribution, transportation and dynamics of antibiotics and ARGs in landfills remain largely unknown. In the present study, 3 antibiotics – sulfamethoxazole (SMX), tetracycline (TC), and oxytetracycline (OTC) – and their related ARGs (*sulI* and *tetO*) were quantified in 51 refuse samples from different depths at 8 locations within a large-scale landfill in central China. The average concentration of OTC was the highest, up to 100.9 ± 141.81 μg/kg (dw, n = 48), followed by TC (63.8 ± 37.7 μg/kg, n = 40), and SMX (47.9 ± 8.1 μg/kg, n = 30). Both *sulI* and *tetO* were detected in all samples. Of the ARGs, *sul1* (−3.06 ± 1.18, *n* = 51, $\log_{10}$ ARGs/16SrDNA) was more abundant than *tetO* (−4.37 ± 0.97) in all refuse samples ($p < 0.05$). Both *sulI* and *tetO* negatively correlated to refuse age, suggesting they are attenuated during landfill stabilization. OTC and TC positively correlated to *tetO* (r = 0.41, $p < 0.01$) and *sulI* (r = 0.29, $p = 0.04$), respectively. Chemical conditions (e.g. moisture content and nitrate concentrations) within the refuse correlated to antibiotics and ARGs suggesting environmental factors impact the distribution

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of antibiotics and ARGs in landfill matrix. This study is the first comprehensive in situ landfill study to connect the concentrations of antibiotic residues to ARGs.

1. Introduction

Each year the use and disposal of antibiotics results in the release of large amounts of antibiotics into environment, raising concerns over the risk of promoting antibiotic resistance (Baquero et al., 2008). Antibiotics enter into the environment through a variety of pathways, including: discharge from municipal sewer systems (Munir et al., 2011) and reclaimed wastewater (Jones-Lepp et al., 2010), the application of biosolids onto agricultural fields (Xu et al., 2009), and via aquaculture (Hoa et al., 2011). The most prevalent pathway results from consumer consumption, and subsequent excretion, of antibiotics which are then introduced into the municipal sewer systems and released into surface waters following processing at a wastewater treatment plant (WWTP) (Graham et al., 2010). In the United Kingdom, public health experts have recommended that unused medication be flushed down the toilet to protect children and animals from accidental poisoning (Bound and Voulvoulis, 2005). Only recently has the impact of this method of disposal been considered. With increasing evidence that pharmaceuticals enter the aquatic environment via wastewater effluent at alarming rates (Kolpin et al., 2002), many in the U.S. have begun to recommend disposing of unused pharmaceuticals, including antibiotics, in municipal landfills (Musson and Townsend, 2009; Quality MDoE, 2007). The new policy will undoubtedly result in increased amounts of drugs entering MSW landfills (Musson and Townsend, 2009). In China, the unused and unwanted drugs have been listed as hazardous materials, but large amount of drugs enters into landfill by household waste directly due to the absence of regulation. Landfills are recognized as a significant source of new and emerging pollutants, including antibiotics (Musson and Townsend, 2009). For example, high concentrations (10–1000 μg/L) of sulfonamides have been detected in the groundwater down gradient of a landfill site in Denmark (Holm et al., 1995). Sulfonamide has also been detected in concentrations up to 28.7 ng/L (mean) in groundwater proximate to a landfill in Guangzhou, China (Peng et al., 2014). In another recent study, high concentrations of sulfonamide (402 ± 704 ng/L, $n = 42$) was observed in landfill leachate in Shanghai, China. Despite these studies, the extent of antibiotic residues present in landfills has not been fully characterized.

The presence of antibiotics in the environment results in the formation of ARGs. ARGs have recently been identified as emerging contaminants and are becoming a global human health challenge (Pei et al., 2006). Regardless of whether present in living or dead cells, ARGs can persist in the environment even after the selective pressure responsible for their formation has been removed. Horizontal gene transfer (HGT) accounts for the major mechanism for ARGs sharing between microbes (Courvalin, 1994), leading to the occurrence of multiple drug resistance (MDR) (Mazel, 2004). Increasing evidences shows that ARGs exists in various environments, including sediments (Luo et al., 2010), rivers (Garcia-Armisen et al., 2011; Luo et al., 2010), all forms of sewage (influent, effluent, and activated sludge) (Chen and Zhang, 2013; Munir et al., 2011), swine farms (Zhu et al., 2013), and soil (Wu et al., 2010). High concentrations of ARGs have also been detected in landfill leachate. For instance, in two recent studies, ARGs (*tetW*, *sulI*, and *sulII*) were detected at transfer stations and in landfill leachate within a landfill in China (Wang et al., 2015; Wu et al., 2015). Similar to antibiotics, ARGs have not been comprehensively investigated within a landfill site.

Accumulated resistance developed by antibiotics and other factors (e.g., metals and contaminants) contributes to the prevalence of ARGs in landfills. The constant release of antibiotics and ARGs via landfill leachate into the environment is likely to have negative health implications. This is particularly true for landfills that do not have engineered/geomembrane liners and leachate collection systems (Song et al., 2009). To better understand the fate and transport of antibiotics and ARGs from landfills, a full, simultaneous characterization of both within landfills is required.

To address this critical knowledge gap, the concentration of antibiotics and related ARGs was determined for 51 refuse samples collected from a large-scale landfill, Jiangchungou (JCG), located at city Xi'an, China. The presence of 3 antibiotics (Table S1) – sulfamethoxazole (SMX), tetracycline (TC) and oxytetracycline (OTC) – and 2 related ARGs – sulfonamide resistance genes (*sulI*) and tetracycline resistance genes (*tetO*) – were measured. Sulfonamide and tetracycline are two classes of antibiotics that are widely used in China (Zhang et al., 2015). Some of sulfonamide and tetracycline antibiotics (Wu et al., 2015) and the related ARGs (*sulI* and *tetO*) (Wang et al., 2015; Wu et al., 2015) have been detected in landfill leachate. However, the concentrations of these constituents in refuse have not been described. To address this gap in knowledge, this study quantified the in situ concentration of SMX, TC, and OTC and *sulI* and *tetO*. Eight essential chemical parameters of refuse were also measured. Finally, the relationship between ARGs, antibiotics and chemicals parameters were investigated. To our knowledge this is the first field-study to simultaneously report the occurrence of antibiotics and ARGs within a landfill site.

2. Materials and methods

2.1. Descriptions of sampling sites and sampling methodology

The JCG sanitary landfill is located at east of City Xi'an, China (Fig. 1). Xi'an is semi-arid with 553 mm precipitation per year (China Meterology Administration, CMA, 2012). The valley-based landfill has a total waste volume of 20×10^6 m^3. The landfill was opened in 1994 and received an average of 5500 t of refuse per day. The landfill is comprised of three units, designated units A, B, and C based on the landfilling age. Units A and B have been inactive (i.e. no longer receiving waste) for 15 and 10 years, respectively. However, the top of unit B received additional refuse in 2012, increasing the height 4 m with fresh refuse. Unit C is an active landfill unit. The average age of refuse at the time of sampling in unit A, B, and C was 15–20, 0.3–15, and 0–5 years, respectively. Information regarding when and where refuse was placed in corresponding cells is well documented. Therefore, it was possible to associate refuse samples to the time at which each they arrived at the landfill.

A total of 51 samples were collected from 8 locations (designated P1 to P8) at depths of 0.8–35.0 m from the 3 units (Fig. 1). Samples were collected from August through October 2013 (Table S2). The age of refused collected ranged from 0.3 to 20 years.

Samples were collected by extracting refuse cores following the Chinese National Standard GB: Code for the Investigation of Geotechnical Engineering (GB50021-2001) and the Technical Code for the Geotechnical Engineering of Municipal Solid Waste Sanitary Landfills (CJJ176-2012). Accordingly, an engineering drill with a steel sleeve XY-100 (bottom inner diameter of 110 mm and upper inner diameter of 150 mm; Aicheng Co, Shanghai, China) was pressed into the landfill up to maximum 35 m resulting in refuse being compacted into the steel sleeve. The refuse in the steel sleeve was then separated into 20 cm long cores and stored in sterilized 20 cm steel sleeves (inner diameter: 150 mm, height: 200 mm). The sleeve was then sealed tightly and stored on ice during transport back to the laboratory. The average depth of refuse cores were recorded. For example, refuse collected from a depth of 0.8 m to 1.0 m resulted in a 0.9 m sample.