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Prevalence of fluoroquinolone, macrolide and sulfonamide-related resistance genes in landfills from East China, mainly driven by MGEs

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ABSTRACT

Landfills are one of the most important reservoirs of antibiotic resistance genes (ARGs), and ARG pollution in landfills has been well investigated. However, the various factors contributing to the widespread prevalence of ARGs in landfills have rarely been explored. Here, we quantified three classes of antibiotics, six kinds of heavy metals, eight types of ARGs, and five varieties of mobile genetic elements (MGEs) in refuse samples from 10 landfills in Zhejiang Province, China. Compared with sulfonamides and macrolides, fluoroquinolones were present at much higher concentrations in all refuse samples, reaching a concentration of 1406.85 μg/kg in the Jiaxing region. The relative abundances of *qnrD*, *qnrS*, *mexF*, *ermA*, *ermB*, *mefA*, *sul1*, and *sul2* in most landfills were $> 10^{-4}$ copies per 16S rRNA, suggesting the presence of highly contaminated ARGs. No significant correlations between most target antibiotics and their corresponding ARGs were found. Variation partitioning analysis indicated that MGEs could be the determining factor in the spread of ARGs in landfills. This research not only reveals high levels of ARGs and the ubiquitous presence of antibiotics in refuse, but also provides guidance for controlling the spread of ARGs in landfills.

1. Introduction

Antibiotics play important roles in the development of medicine, modern agriculture, and animal husbandry (Price et al., 2015; Zhou et al., 2013). As one of the largest antibiotic producers and consumers in the world, approximately 248,000 and 162,000 tons of antibiotics are produced and consumed, respectively, per year in China (Zhang et al., 2015a). These antibiotics are ultimately released into various environments and increase the prevalence of both antibiotic-resistant bacteria and antibiotic resistance genes (ARGs). The emergence of multidrug-resistant "superbugs" has significant implications for mortality rates worldwide, with the number of deaths caused by antibiotic-resistant bacterial infections expected to exceed 10 million by 2050 (Release, 2017). As one of the most severe ecological risks, ARG pollution has attracted a significant amount of attention worldwide (Andersson and Hughes, 2014; Szabo et al., 2017).

Landfills are widely used for municipal solid waste (MSW) disposal (Ying et al., 2019). It is estimated that worldwide, landfills annually receive approximately 350 million tons of MSW (Wu et al., 2017). As a result of poor management of antibiotic use, considerable amounts of expired antibiotics end up in landfills. For example, antibiotic concentrations > 1 mg/kg were detected in refuse from Shanghai, while antibiotic concentrations in leachates from Hangzhou exceeded 100 mg/L (Wu et al., 2017; You et al., 2018). Both of these concentrations are high enough to promote the evolution of antibiotic resistance in bacteria. In addition to antibiotics, various heavy metals and organic pollutants are prevalent in landfills, and may also exert stress on bacteria and induce the co-selection of antibiotic resistance (Sun et al., 2016). Landfills are highly important half-open ecosystems; thus, the abundance of ARGs in bacterial communities at these sites could affect their corresponding abundance in other ecosystems. It is therefore imperative that we investigate ARG pollution of landfills.

Abbreviations: ARGs, Antibiotic resistance genes; FQs, Fluoroquinolones; MLs, Macrolides; SAs, Sulfonamides; MSWs, Municipal solid wastes; MGEs, Mobile genetic elements; NH_4^+–N, Ammonia nitrogen; NO_3^-/NO_2^-–N, Nitrate/nitrite; TOC, Total organic carbon; DOC, Dissolved organic carbon; TP, Total phosphorus; SO_4^{2-}, Sulfate; Cl^-, Chloride

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ARG pollution of landfills has already been well studied. A study from central China showed a high abundance of sulfamethoxazole-related ARGs but a low abundance of tetracycline-related ARGs in landfill samples (Song et al., 2016), while β-lactam- and fluoroquinolones-associated ARGs were prevalent in landfill samples from Shanghai (You et al., 2018). Overall, most studies have focused on mega-cities in China, with few studies investigating ARG pollution in small- and medium-sized cities, which may have a more complex waste input. This complex input could induce co-selection of antibiotic resistance in bacteria. However, how various factors, including environmental conditions, antibiotics, heavy metals, and bacterial interactions, affect the prevalence of ARGs in small- and medium-sized landfills is not well understood, which limits our ability to control ARG pollution in these ecosystems.

Therefore, in the current study we collected samples from 10 different landfills from small- and medium-sized cities in East China. We examined the abundance of ARGs as well as the concentrations of antibiotics and heavy metals in the samples. Moreover, the relationships between various factors, including antibiotic concentrations (fluoroquinolones, macrolides, and sulfonamides), environmental factors (pH and moisture), nutrient concentrations (dissolved organic carbon (DOC), NH_4^+–N, NO_3^-/NO_2^-–N, and total phosphorus (TP)), and heavy metal contents (Pb, Zn, Cu, Cd, Cr and Ni), and the prevalence of ARGs were analyzed. The effects of bacterial interactions on the abundance of ARGs were also evaluated by exploring the relationships between mobile genetic elements (MGEs) and ARGs. This study not only enriches our knowledge on ARG pollution, but also provides guidance on controlling the spread of ARGs in landfills.

2. Materials and methods

2.1. Sample collection and pretreatment

Refuse samples were collected from 10 small- and medium-sized landfills in Zhejiang Province, China (Fig. 1). The locations (latitude and longitude), capacities, and ages of all landfills are listed in Table S1. At each location, five samples were collected within a 100-m^2 area at a depth of 1.5 m. Approximately 10 kg of refuse from each of these five sampling sites were combined into one sample per location. The samples were then shipped back to the laboratory on dry ice. All samples were collected from July to November 2018 (Table S1).

Fig. 1. Locations of the sampled landfills in Zhejiang Province, China.

Each sample was divided into three portions. One portion was used for physical and chemical index determinations, including pH, moisture, DOC, NH_4^+–N, NO_3^-/NO_2^-–N, TP, Cl^-, SO_4^{2-}, and heavy metals, while the second portion was stored at −40 °C for the detection of antibiotics. The third portion was stored at −80 °C for DNA extraction. Large particles, such as plastic bags and sandstones, were removed. To reduce potential bias caused by the heterogeneous composition of municipal solid waste in subsequent analyses, refuse samples were lyophilized (typically ~100-g aliquots) using a freeze-dryer. The frozen samples were then ground and passed through a 0.15-mm sieve.

2.2. Physicochemical analysis

Physicochemical characterization of all samples was performed according to standard methods for soil chemistry analysis (Lu, 2000). The pH was measured from refuse-water suspensions (1:5, v/v). Refuse moisture was measured gravimetrically. NH_4^+–N and NO_3^-–N were separately extracted using 2 M KCl and then analyzed by Nessler's reagent spectrophotometry and phenol disulfonic acid colorimetry, respectively (Wang et al., 2017). NO_2^-–N concentrations were determined using the Brucine method (Tang et al., 2019). TP was measured using the molybdenum blue spectrophotometric method (Song et al., 2016). DOC was measured using a TOC analyzer (Ying et al., 2019). SO_4^{2-} and Cl^- were quantified using ion chromatography (Liu et al., 2018). All samples were measured at least in triplicate.

2.3. Measurement of antibiotics

Twenty-one antibiotics belonging to three different classes, including fluoroquinolones, macrolides, and sulfonamides, were quantified in this study (Table S3). The antibiotics were extracted using solid-phase extraction with tandem cartridges. The first cartridge was a strong anion exchanger (3 mL/200 mg; Supelco, USA) for cleanup, while the second cartridge was a hydrophile-lipophile balance (6 mL/500 mg; Waters, USA) for antibiotic absorption. Prior to extraction, cartridges were preconditioned with 10 mL of methanol and 10 mL of ultrapure water at a rate of 1 mL/min. The operational flow rate of solid-phase extraction was maintained at 3–5 mL/min. A 1-mL aliquot of the extracted sample was filtered through a 0.22-μm hydrophobic polytetrafluoroethylene membrane and stored at −20 °C. Antibiotic concentrations were measured by ultra-performance liquid chromatography-tandem mass spectrometry. The mobile phase consisted of eluent A (ultrapure water with 0.1% formic acid) and eluent B (acetonitrile). A triple quadrupole tandem mass spectrometer was used in subsequent analyses with a Z-spray electrospray interface (Waters, USA). The liquid phase and mass spectrometry conditions are described in the Supplementary Information (Tables S2–S4).

2.4. Heavy metal analysis

Six frequently-detected heavy metals (Pb, Zn, Cu, Cd, Cr, and Ni) were selected for analysis and measured. In detail, 0.30 g of refuse was mixed with hydrochloric acid, nitric acid, and hydrofluoric acid (1:3:2, v/v/v) and then digested by microwave for 1 h. The digested solutions were then placed on a hot plate for acid scavenging. The resulting mash was rinsed using ultrapure water and resuspended in a final volume of 50 mL. Following filtration through a 0.22-μm aqueous membrane, the heavy metals were quantified using inductively-coupled plasma-atomic emission spectroscopy (novAA800; Jena, Germany) as previously described (Sun et al., 2016).

2.5. DNA extraction

DNA was extracted as described previously (Staley et al., 2012), with slight modification. Briefly, 0.25 g of refuse was vortexed in 5 mL

of phosphate-buffered saline for 2 min. The mixture was centrifuged at 12,000 × *g* for 10 min and then washed three times with ultrapure water. The resulting precipitate was used for DNA extraction. Total DNA was extracted from each sample using a Fast DNA Kit for soil (MP Biomedicals, USA) according to the manufacturer's instructions. Extracted DNA with an $OD_{260/280}$ between 1.7 and 2.0 was used for subsequent analyses. The qualified DNA samples were stored at −80 °C for quantitative polymerase chain reaction (qPCR) analysis.

2.6. High-throughput real-time qPCR

Eight targeted ARGs, including three fluoroquinolone ARGs (*qnrS*, *qnrD*, and *mexF*), three macrolide ARGs (*mefA*, *ermA*, and *ermB*), and two sulfonamide ARGs (*sul1* and *sul2*), were analyzed. In addition, five MGEs (*intl1*, *intl2*, *tnpA01*, *tnpA02*, and *tnpA03*) were examined. A TB Green Premix Ex Taq II (Tli RNaseH Plus) Kit (Takara, Japan) was used for high-throughput real-time qPCR amplification. Each 10-μL reaction contained 1 μL of template DNA, 5 μL of TB Green Premix Ex Taq II (Tli RNaseH Plus), 3 μL of double-distilled water, 0.4 μL of each primer, and 0.2 μL of ROX reference dye. All primer sets were designed and synthesized by Wcgene Biotechnology Corporation (China). Primer sequences are provided in Table S5.

qPCR analysis was performed under the following conditions: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s, with a final extension at 60 °C for 10 min. A melt curve was automatically generated by the StepOnePlus Real-Time PCR System (Applied Biosystems, USA), with a cycle threshold (C_T) of 40 used as the detection limit.

The $2^{-\Delta CT}$ method (Schmittgen and Livak, 2008) was used to calculate the relative abundance of each of the ARGs, where $\Delta C_T = C_{T\ (Genes)} - C_{T\ (16S\ rRNA)}$, C_T is the threshold cycle, and $C_{T\ (Genes)}$ and $C_{T\ (16S\ rRNA)}$ represent the threshold cycles of the targeted ARGs/MGEs and 16S rRNA gene, respectively.

2.7. Statistical analysis

Pearson correlation analyses were conducted in SPSS version 23.0 (IBM Corporation, USA). Variation partitioning analysis was performed using R software version 3.2 with the vegan package. Redundancy analysis was performed using Conoco version 5.0 (Microcomputer Power, USA) (Yang et al., 2014). GraphPad Prism version 8.0.1 (GraphPad Software Corporation, USA) was used to generate for bar charts. All data were expressed as the means ± standard deviation (S.D.). Statistical significance was defined by 95% confidence intervals ($p < 0.05$).

3. Results and discussion

3.1. Profiles of the investigated landfills

Ten landfills distributed across 10 small- and medium-sized cities in Zhejiang Province, China, were investigated in this study. Excluding those from Ningbo and Wenzhou, all landfills were situated in valleys. All landfills in this study were classified as anaerobic, and predominantly received domestic garbage, kitchen waste, clinical refuse, and activated sludge. As shown in Table 1, the refuse from these landfills was slightly alkaline, and the moisture content ranged from 33.61% to 59.19%. The DOC, NH_4^+–N, NO_3^-/NO_2^-–N, and TP concentrations varied considerably across the different landfills. Generally, the DOC, NH_4^+–N, NO_3^-/NO_2^-–N, and TP concentrations in refuse samples from Jiaxing, Hangzhou, Shaoxing, and Taizhou were higher than those in samples from the other regional landfills.

Three classes of antibiotics, including fluoroquinolones, macrolides and sulfonamides, were investigated in the landfill samples. As shown in Fig. 2, all three classes of antibiotics were detected in all sampled landfills. Fuoroquinolone concentrations were higher than those of macrolides and sulfonamides. For example, in the Jiaxing region, fluoroquinolones were detected at a concentration of 1.4 mg/kg, while macrolide and sulfonamide antibiotics were only present at μg/kg concentrations. Interestingly, all three classes of antibiotics were present at significantly higher concentrations in samples from the Jiaxing region compared with those from other regions. This is likely the result of rapid development of the livestock industry in this region (Zhu et al., 2013). Previous studies have shown that antibiotic concentrations are generally < 50 μg/kg in sediment (Chen and Zhou, 2014; Luo et al., 2011) and soil (Wang et al., 2014). Similarly, antibiotic concentrations in surface water (Li et al., 2014; Zhang et al., 2012) and samples from wastewater treatment plants (Wang et al., 2019) are generally in the ng/L to μg/L range. Landfills receive unwanted and unused drugs, illegal clinical waste, animal feces, used diapers, and toilet paper from clinics, hospitals, and households (Eggen et al., 2010; Threedeach et al., 2012; Zhao et al., 2018). As a result, antibiotics are often present at much higher concentrations (μg/kg to mg/kg) in landfills compared with other ecosystems. Thus, landfills are considered the main repositories for antibiotics (Song et al., 2016).

Our previous study showed that Cu, Cd, Cr, Zn, Ni, and Pb are frequently detected in landfills (Long et al., 2011). Thus, these six heavy metals were examined in the current study. As shown in Table 2, Cu, Cr, Zn, and Pb were detected at concentrations > 100 mg/kg in all of the landfills. The highest concentrations of Cu and Zn were detected in the Jinhua and Taizhou regions, with concentrations of both metals exceeding 1000 mg/kg in these samples. In comparison, Cr and Pb were abundant in samples from Wenzhou, with concentrations of 1179.71 mg/kg and 329.58 mg/kg, respectively. The high Cu and Zn concentrations in Taizhou possibly result from the disposal of electronic items, as Taizhou is one of the major electronic waste recycling and disposal centers in China (Tang et al., 2010). High concentrations of Cr and Pb in the Wenzhou samples are likely attributable to the active leather working industry in the area (Song et al., 2012; Zhang and Zhang, 2007). Based on Chinese environmental quality standards for soil (Chinese National Standard: GB 15,618–1995; Cu, 100 mg/kg; Zn, 300 mg/kg; Cr, 250 mg/kg), this level of heavy metal contamination in the landfills is considerable, and possibly induces stress that could contribute to the evolution of antibiotic resistance (Wu et al., 2015; Zhang et al., 2016).

3.2. Abundances of target ARGs in landfill refuse

We examined the abundance of eight different ARGs in the current study, including fluoroquinolone-related ARGs (*qnrD*, *qnrS*, and *mexF*), macrolide-related ARGs (*ermA*, *ermB*, and *mefA*), and sulfonamide-related ARGs (*sul1* and *sul2*). As shown in Fig. 3, sulfonamide-related ARG concentrations were generally higher than those of both fluoroquinolone- and macrolide-associated ARGs. The relative abundances of *sul1* and *sul2* ranged from 2.19×10^{-3} to 1.04×10^{-1} copies/16S rRNA and from 4.61×10^{-3} to 2.88×10^{-1} copies/16S rRNA, respectively. Meanwhile, fluoroquinolone-related ARGs (*qnrD*, *qnrS*, and *mexF*) and macrolide-related ARGs (*ermA*, *ermB*, and *mefA*) ranged in concentration from 8.19×10^{-6} to 1.27×10^{-1} copies/16S rRNA and from 4.94×10^{-6} to 2.16×10^{-1} copies/16S rRNA, respectively. The abundances of the sulfonamide-related ARGs (*sul1* and *sul2*) were approximately one to two orders of magnitude higher than those of the fluoroquinolone-related (*qnrD*, *qnrS*, and *mexF*) and macrolide-related (*ermA*, *ermB*, and *mefA*) ARGs.

Typically, the relative abundance of ARGs in unpolluted environments ranges from 10^{-8} to 10^{-6} copies/16S rRNA (Wang et al., 2015), whereas concentrations in highly contaminated sites are often several orders of magnitude higher (e.g., 10^{-4}) (Graham et al., 2011; Munir et al., 2011). In this study, the abundances of *qnrD*, *qnrS*, *mexF*, *ermA*, *ermB*, *mefA*, *sul1*, and *sul2* in most landfills were greater than 10^{-4} copies/16S rRNA. Previous studies suggest that the relative abundance of *qnrS* and *qnrD* in soil is generally below 10^{-4} copies/16S rRNA

Table 1
Physicochemical parameters in landfill refuse samples.

Sample Sites	Moisture (%)	pH	Nutrients (mg/kg-dry refuse)					Cl^- (g/kg)	SO_4^{2-} (g/kg)
			NH_4^+–N (mg/kg)	NO_3^-–N (mg/kg)	NO_2^-–N (mg/kg)	DOC (mg/kg)	TP (mg/kg)		
HuZ	42.59 ± 11.76	7.82 ± 0.29	22.99 ± 16.28	51.72 ± 18.22	3.78 ± 0.72	878.40 ± 569.01	1382.14 ± 846.81	0.79 ± 0.36	8.39 ± 6.82
JH	51.16 ± 10.83	7.91 ± 0.01	293.85 ± 66.42	37.36 ± 19.30	7.17 ± 0.74	1238.20 ± 5.09	933.33 ± 85.47	2.18 ± 0.65	3.03 ± 0.04
JX	59.19 ± 7.33	8.08 ± 0.44	19.58 ± 15.63	102.04 ± 11.97	5.00 ± 3.67	6292 ± 407.49	2704.58 ± 198.46	4.99 ± 2.06	3.33 ± 0.99
LS	51.84 ± 6.01	8.43 ± 0.13	219.31 ± 37.84	87.68 ± 7.24	1.85 ± 0.53	1402.80 ± 104.88	1890.85 ± 102.33	1.02 ± 1.21	2.33 ± 1.03
NB	47.20 ± 4.84	8.04 ± 0.03	122.74 ± 2.75	39.63 ± 6.46	2.31 ± 0.54	356.40 ± 8.77	3318.95 ± 338.11	1.71 ± 0.26	1.95 ± 0.28
QZ	55.98 ± 24.24	8.09 ± 0.24	296.85 ± 83.79	48.87 ± 5.43	12.18 ± 0.67	899.07 ± 58.14	1475.82 ± 397.46	0.79 ± 0.53	2.89 ± 1.28
WZ	54.43 ± 3.61	7.91 ± 0.22	68.83 ± 3.52	120.32 ± 33.46	8.54 ± 0.60	1410.20 ± 201.95	3822.22 ± 462.16	0.55 ± 0.37	3.86 ± 0.58
HaZ	46.50 ± 8.77	7.24 ± 0.12	132.51 ± 35.78	24.95 ± 1.33	25.40 ± 3.22	9164.93 ± 339.05	5265.94 ± 249.01	4.62 ± 0.99	4.35 ± 2.08
SX	57.55 ± 9.75	7.56 ± 0.13	289.72 ± 59.58	3.90 ± 1.13	1.47 ± 0.29	4118.67 ± 30.88	3611.64 ± 369.73	4.16 ± 0.58	15.93 ± 5.36
TZ	33.61 ± 1.84	7.96 ± 0.02	210.67 ± 6.61	14.76 ± 0.31	0.95 ± 0.24	2066.37 ± 148.39	6722.46 ± 70.77	6.30 ± 0.84	10.12 ± 1.86

All data shown are mean ± standard deviation.

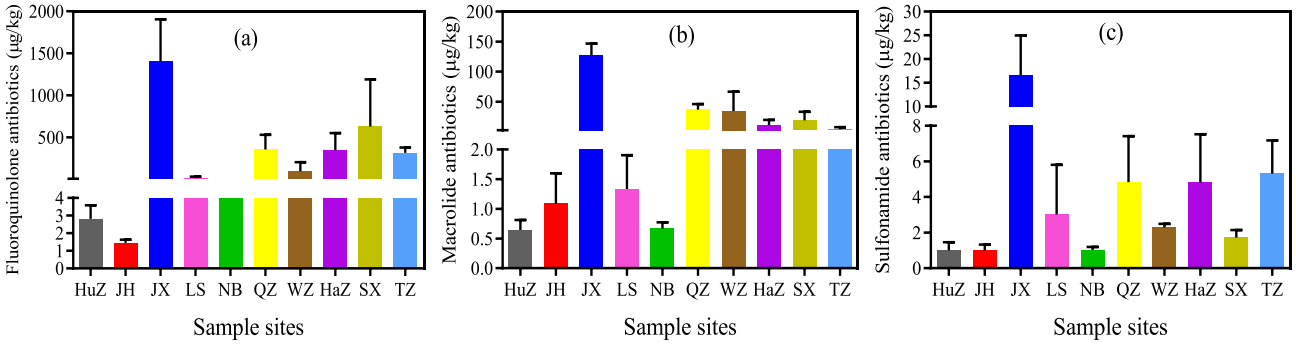

Fig. 2. Total concentrations of (a) fluoroquinolone (FQ) antibiotics, (b) macrolide (ML) antibiotics, and (c) sulfonamide (SA) antibiotics in landfill refuse samples.

Table 2
Levels of heavy metals in refuse from different landfills.

Sample Sites	Cu (mg/kg)	Cd (mg/kg)	Cr (mg/kg)	Zn (mg/kg)	Ni (mg/kg)	Pb (mg/kg)
HuZ	103.99 ± 56.26	10.63 ± 3.81	669.81 ± 129.31	553.35 ± 273.61	101.03 ± 70.62	154.44 ± 92.31
JH	1728.00 ± 60.80	2.62 ± 0.52	786.92 ± 88.79	1773.36 ± 182.42	196.36 ± 7.12	240.95 ± 77.95
JX	243.95 ± 68.04	6.36 ± 0.60	855.02 ± 100.88	1407.32 ± 398.19	82.09 ± 7.88	283.22 ± 58.03
LS	267.18 ± 54.54	9.16 ± 0.31	495.75 ± 72.60	1031.19 ± 248.08	110.51 ± 51.73	294.91 ± 109.25
NB	756.83 ± 2.44	6.77 ± 0.01	168.48 ± 5.68	880.11 ± 41.11	256.67 ± 1.22	139.88 ± 5.87
QZ	234.34 ± 76.85	2.52 ± 0.11	208.90 ± 59.23	637.78 ± 185.50	163.26 ± 16.28	156.35 ± 53.61
WZ	469.71 ± 65.47	5.31 ± 0.04	1179.71 ± 177.60	1120.03 ± 56.80	120.89 ± 40.23	329.58 ± 119.97
HaZ	154.39 ± 48.97	5.91 ± 0.93	432.57 ± 28.39	788.59 ± 429.59	11.95 ± 6.05	119.08 ± 21.83
SX	181.14 ± 37.76	6.07 ± 0.24	265.41 ± 103.08	1052.31 ± 257.46	71.93 ± 13.82	186.92 ± 14.29
TZ	1074.79 ± 84.79	4.88 ± 2.22	232.30 ± 21.28	1603.69 ± 245.17	33.10 ± 7.65	227.54 ± 52.03

All data shown are mean ± standard deviation.

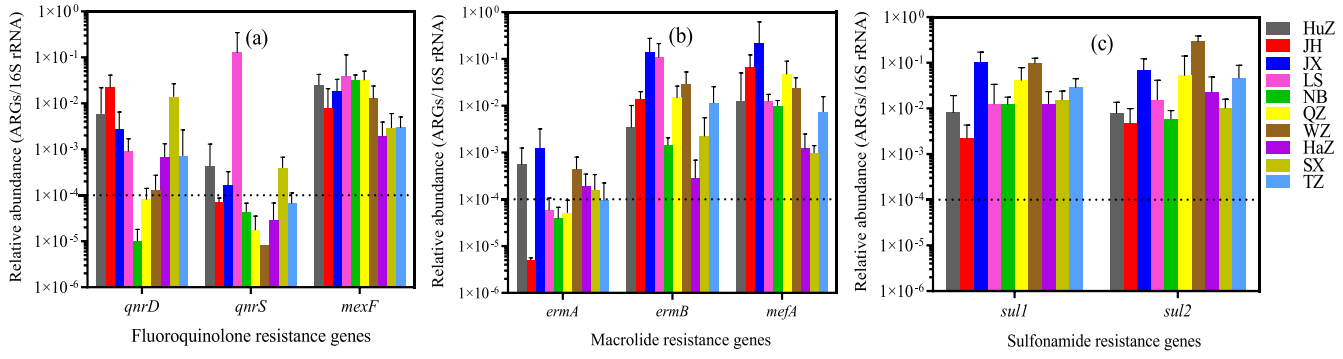

Fig. 3. Distribution of (a) fluoroquinolone (FQ) ARGs, (b) macrolide (ML) ARGs, and (c) sulfonamide (SA) ARGs in landfill refuse samples.

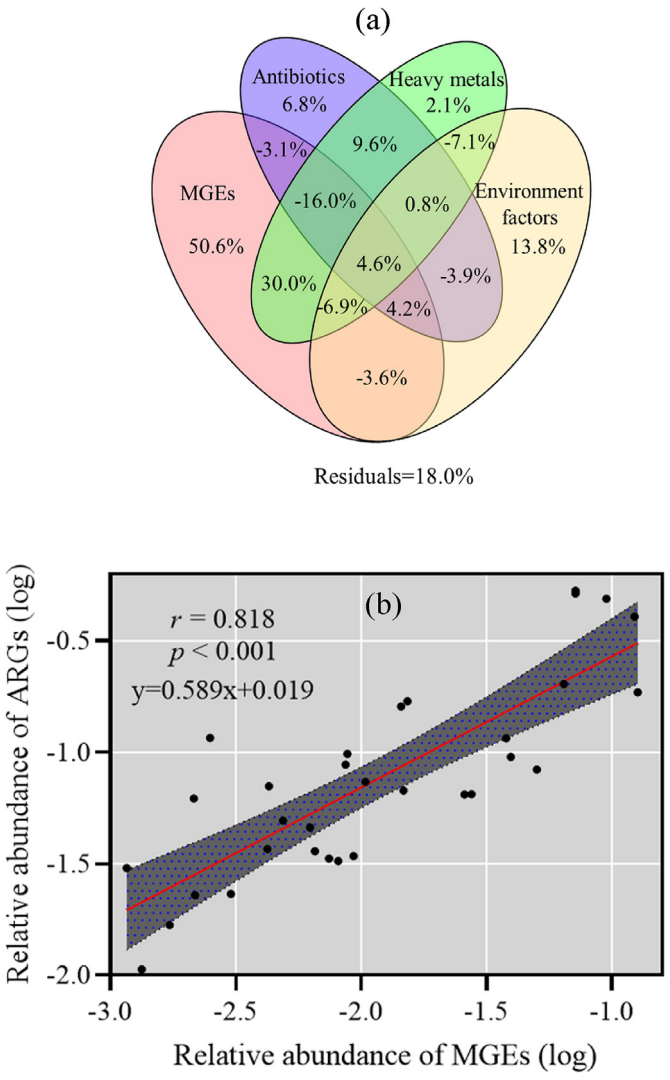

Fig. 4. (a) Variation partitioning analysis differentiating the effects of mobile genetic elements (MGEs), environmental factors, antibiotics, and heavy metals on the alteration of target ARGs in landfill refuse. (b) Correlation between the relative abundance of MGEs and the relative abundance of ARGs.

(Zhang et al., 2015b), while *ermA* and *ermB* are present at concentrations below 10^{-5} copies/16S rRNA in air (Li et al., 2018). In addition, the abundance of *sul1* ranged from 10^{-6} to 10^{-2} copies/16S rRNA in sediments, while *sul2* abundance was less than 10^{-6} copies/16S rRNA in surface water (Ohore et al., 2019). Compared with other ecosystems, landfills show a much higher level of ARG pollution. A considerable amount of livestock manure, activated sludge, and illegal medical waste, all of which contain high concentrations of ARG-carrying bacteria, is disposed in landfills (Ju et al., 2016; Wu et al., 2018). In addition, high concentrations of heavy metals and organic pollutants contribute to the co-selection of the antibiotic resistance (Duan et al., 2019; Sun et al., 2016). Together, these factors significantly contribute to the higher levels of ARG pollution in landfills compared with other ecosystems.

We observed significant variation in the prevalence of the different ARGs among the different regions. As shown in Fig. 3a, *qnrS* and *mexF* were most abundant in refuse from the landfill in the Lishui region, followed by samples from Huzhou, Shaoxing, and Jiaxing. The average relative abundances of *qnrS* and *mexF* in Lishui were 1.27×10^{-1} and 3.90×10^{-2} copies/16S rRNA, respectively. As shown in Fig. 3b, the greatest abundance of macrolide-associated ARGs (*ermA*, *ermB*, and *mefA*) was observed in refuse from the Jiaxing landfill. The average relative abundances of *ermA*, *ermB*, and *mefA* were 1.24×10^{-4}, 1.41×10^{-1}, and 2.16×10^{-1} copies/16S rRNA, respectively. As shown in Fig. 3c, the greatest abundances of sulfonamide-related ARGs *sul1* and *sul2* occurred in the Jiaxing (1.04×10^{-1}) and Wenzhou (2.88×10^{-1}) regions, respectively. Overall, the relative abundances of *sul1* and *sul2* exceeded 10^{-2} copies/16S rRNA in all regions except for Huzhou, Jinhua, and Ningbo.

As demonstrated in previous studies, landfills are the main repositories for ARGs (Su et al., 2019). Of the genes investigated in the current study, the abundances of fluoroquinolone-related ARGs *qnrD*, *qnrS*, and *mexF* in samples from the Jiaxing and Lishui regions were approximately one order of magnitude higher than those in samples from Guiyang, Suzhou, and Nanjing (You et al., 2018). In comparison, macrolide-associated ARGs *ermA*, *ermB*, and *mefA* and sulfonamide-related ARGs *sul1* and *sul2* were three-to five-fold more abundant in samples from the Jiaxing and Wenzhou regions compared with those from the Chongqing and Taiyuan landfills (Wang et al., 2015). Compared with mega-cities, small- and medium-sized cities tend to show higher levels of ARG pollution in landfills. The more complex refuse sources in these areas possibly account for the increased ARG pollution of landfills, as multiple types of pollutants can induce the co-selection of antibiotic resistance (Sun et al., 2016). Notably, using Lishui as an example, the concentrations of fluoroquinolones were low, while their corresponding ARG concentrations were very high. This suggested that ARG abundances were not always consistent with their corresponding antibiotic concentrations (You et al., 2018; Wu et al., 2015). Therefore, to better understand the occurrence of ARGs in landfills, factors contributing to their abundance should be further explored.

3.3. Contributions of various factors to the abundances of ARGs

In a previous study, many factors were shown to possibly affect the spread or occurrence of ARGs in landfills (Wu et al., 2015). Thus, the contributions of these factors, including MGEs (integrons and transposons), environmental factors (pH, moisture, DOC, and NH_4^+–N, NO_3^-/NO_2^-–N, TP, SO_4^{2-}, and Cl^- concentrations), antibiotics (fluoroquinolones, macrolides, and sulfonamides), and heavy metals (Cr, Cd, Pb, Cu, Zn, and Ni), were individually examined by variation partitioning analysis. As shown in Fig. 4a, the main factor contributing to the occurrence of ARGs was MGEs, with a contribution of 50.6%. Meanwhile, environmental factors, antibiotics, and heavy metals accounted for 13.8%, 6.8%, and 2.1% of the abundance, respectively.

Although bacterial community shifts rather than MGEs are the major factors shaping the antibiotic resistome in soil and groundwater (Chen et al., 2016, 2017), most studies have shown that MGEs are the determining factors in ARG pollution of landfills (Wu et al., 2018; You et al., 2018). Consistent with these studies, we also found that MGEs had the greatest impact on ARG abundance in refuse samples. In addition, there were significant positive correlations between the relative abundance of ARGs and the relative abundance of MGEs ($r = 0.818$, $p < 0.001$) (Fig. 4b). Compared with soil and groundwater, refuse contains sufficient organic matter to sustain bacterial growth. Under conditions of sufficient nutrition, horizontal gene transfer plays an important role in ARG abundance within a relatively fixed bacterial population (Zhou et al., 2018).

As the most important factor contributing to the occurrence of ARGs, the correlations among various MGEs and ARG occurrence were further analyzed by redundancy analysis. As shown in Fig. 5a, there was a significant positive correlation between *intl1* and *sul1* in refuse ($p < 0.01$). Because *sul1* is embedded within the 3′-conserved region of class 1 integrons (Luo et al., 2014b), *intl1* drives the abundance of *sul1* in various ecosystems (Di et al., 2016; Wu et al., 2015; Yu et al., 2016). *intl2*, the expression of which can be disrupted by the presence of termination codon TAA, has previously only been shown to drive increases in abundance of *aadAla*, *dhfr*, and *sat*. Consistent with these observations, we found that *intl2* was not correlated with the

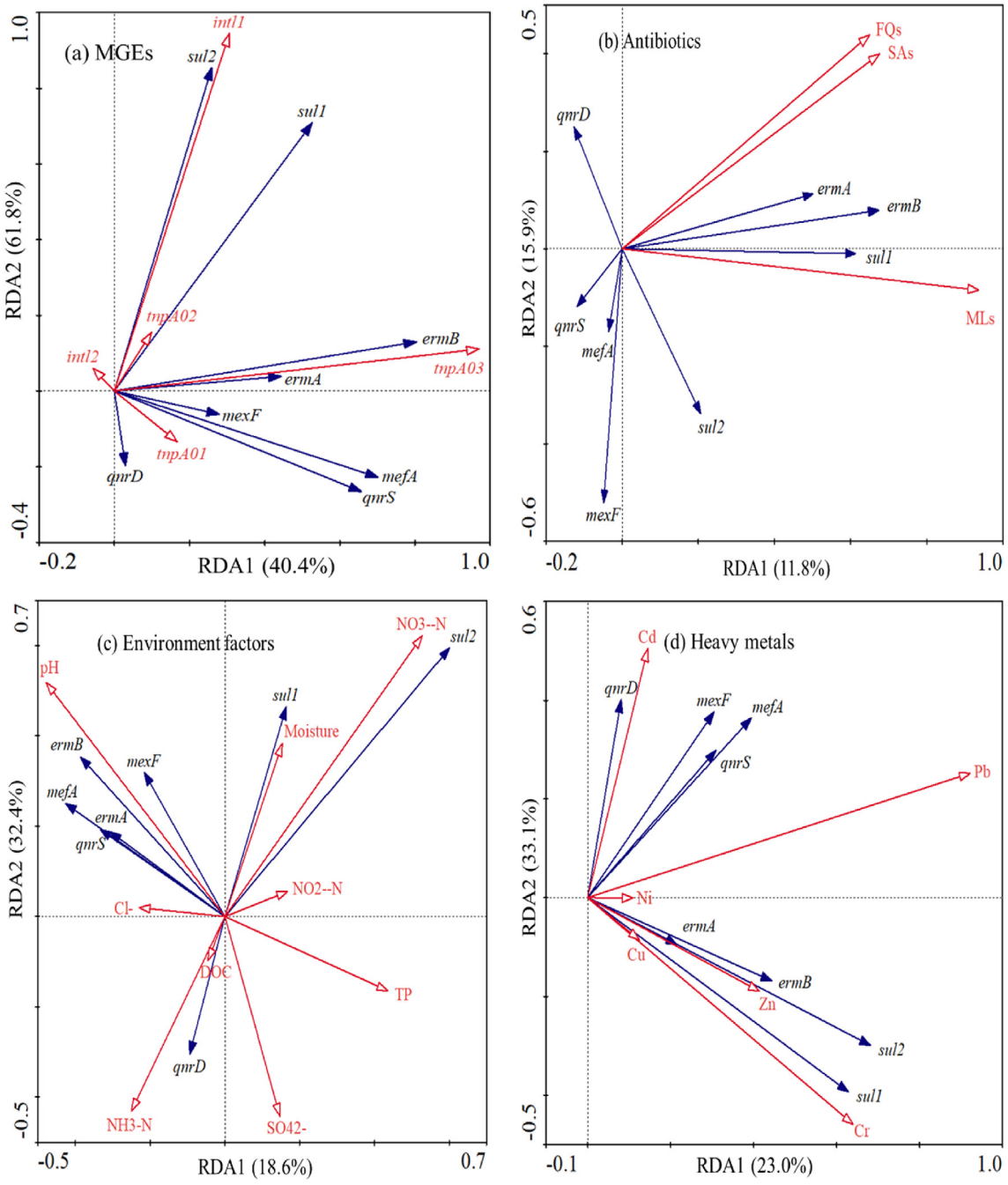

Fig. 5. Redundancy analysis (RDA) of the relationships between (a) MGEs and ARGs, (b) antibiotics and ARGs, (c) environmental factors and ARGs, and (d) heavy metals and ARGs in landfill refuse samples.

abundance of the fluoroquinolone or macrolide resistance genes examined in this study (Li et al., 2017; Zhang et al., 2020). However, transposons were closely associated with the abundance of fluoroquinolone- and macrolide-associated ARGs. In particular, *tnpA03* was significantly positively correlated with the abundance of *qnrS*, *ermA*, *ermB*, and *mefA* ($p < 0.01$, Table S6). Because of their nonspecific integration into the bacterial chromosome, transposons have been correlated with increased abundance of various types of ARGs. In addition, because of their molecule structures, the abundance of *ermA* and *ermB* is often affected by transposons.

Conventional wisdom holds that the prevalence of ARGs is mainly affected by selective pressure from corresponding antibiotics (Shao et al., 2018; Wu et al., 2015). As mentioned above, the contribution of antibiotics in the refuse samples to the abundance of resistance genes was only 6.8% (Fig. 4a). Further, no significant correlations were found between most target antibiotics and their corresponding ARGs (Fig. 5b). Both results suggest that, in contrast to previous thinking, antibiotics do not drive the prevalence of ARGs in landfills (Zhao et al., 2018). The concentrations of most of the target antibiotics in the landfill samples were approximately one to two orders of magnitude lower than the reported minimum inhibitory concentrations in the environment (e.g., sulfamethoxazole, amoxicillin, and erythromycin minimum inhibitory concentrations are 64, 18, and 64 mg/L, respectively), and therefore unlikely to be sufficient to rapidly select for antibiotic resistance (Kibuule et al., 2016; Luo et al., 2014a). Actually, sub-inhibitory concentrations of antibiotics could act as a signal for cell-to-cell communication, which benefits horizontal gene transfer (Jutkina et al., 2018). Under low selective pressure, horizontal gene transfer events are much

more random than those that occur under high stress conditions, and increases in abundance would not necessarily occur in exactly corresponding ARGs (Andersson and Hughes, 2014). Furthermore, the observed significant correlation between *sul1* and *sul2* and the macrolide antibiotics examined in this study ($p < 0.01$, Table S7) provides evidence of co-selection among the ARGs and other classes of antibiotics in landfills (Chen and Zhang, 2013). Both the low selective pressure and co-selection patterns explain the finding that antibiotic stress was not the main factor contributing to the prevalence of ARGs in landfills.

The abundance of ARGs could be affected by environmental factors, which accounted for 13.8% of the observed abundance in the current study. As shown in Fig. 5c, there were significant positive correlations between pH and both macrolide-associated ARGs (*ermA*, *ermB*, and *mefA*) and fluoroquinolone-associated ARGs (*qnrS* and *mexF*) ($p < 0.05$, Table S8). Compared with sulfonamides, fluoroquinolones and macrolides demonstrate higher stability under weak alkaline and solid-phase conditions (k_d (FQs) > k_d (MLs) > k_d (SAs)) (Song et al., 2016; Yu et al., 2016). This stability may promote the generation and spread of macrolide- and fluoroquinolone-related ARGs. Nitrate concentration mainly affected the abundances of *sul1* and *sul2* ($p < 0.01$, Table S8). According to Sun et al. (2016), levels of sulfonamide resistance were higher than levels of macrolide and fluoroquinolone resistance under restricted nitrogen conditions, with *sul1* copy numbers positively correlated with nitrate concentrations (Song et al., 2016). Although the link between nitrate and sulfonamide resistance appears obvious, this mechanism is still worthy of further study.

Our study indicated that heavy metals have less impact on the presence and transfer of ARGs in landfills compared with other factors, which is in line with findings from previous studies (Wu et al., 2017). However, based on additional redundancy analysis (Fig. 5d), there was a close relationship between certain ARGs and specific heavy metals. Some ARGs were impacted by the presence of Zn and Cr. Notably, Cr and Zn were significantly positively correlated with both *sul1* and *sul2* ($p < 0.05$, Table S9). Recent studies suggest that different metals (Cr (VI) and Zn (II)) cause discrete and distinct types of injuries to microbial cells as a result of oxidative stress, protein dysfunction, or membrane damage (Lemire et al., 2013; Zhang et al., 2018). Reactive oxygen species production induced by heavy metals likely contributes to the promotion of conjugative transfer (Zhang et al., 2018), which in turn promotes the spread of ARGs between different bacterial species through horizontal gene transfer.

4. Conclusion

This study revealed that ARGs and antibiotics are highly abundant in landfills across East China. Compared with macrolide and sulfonamide antibiotics, fluoroquinolone antibiotics were present at much higher concentrations in the refuse samples, with a maximum fluoroquinolone concentration of 1406.85 μg/kg recorded in the sample from Jiaxing. The abundances of *qnrD*, *qnrS*, *mexF*, *ermA*, *ermB*, *mefA*, *sul1*, and *sul2* in most of the landfills were $> 10^{-4}$ copies/16S rRNA, indicating high levels of ARG contamination. There were no significant correlations between antibiotics and their corresponding ARGs, while MGEs were identified as the major contributors to the abundance of ARGs in landfills. This study not only reveals that landfills are a reservoir of ARGs, it also provides a theoretical basis and guidance for controlling the spread of landfill ARGs in the future.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.110131.

Author contributions

L.Y., Y.L., D.S., H.F., H.Z., and M.W. designed research; L.Y., Y.L., and Z.L. performed research; L.Y., Y.L., Z.L., and M.W. analyzed data; and L.Y., Y.L., and M.W. wrote the paper.

References

Andersson, D.I., Hughes, D., 2014. Microbiological effects of sublethal levels of antibiotics. Nat. Rev. Microbiol. 12 (7), 465–478.
Chen, H., Zhang, M., 2013. Occurrence and removal of antibiotic resistance genes in municipal wastewater and rural domestic sewage treatment systems in eastern China. Environ. Int. 55, 9–14.
Chen, K., Zhou, J.L., 2014. Occurrence and behavior of antibiotics in water and sediments from the Huangpu River, Shanghai, China. Chemosphere 95, 604–612.
Chen, Q.L., An, X.L., Li, H., Su, J.Q., Ma, Y.B., Zhu, Y.G., 2016. Long-term field application of sewage sludge increases the abundance of antibiotic resistance genes in soil. Environ. Int. 92–93, 1–10.
Chen, Q.L., Li, H., Zhou, X.Y., Zhao, Y., Su, J.Q., Zhang, X., et al., 2017. An underappreciated hotspot of antibiotic resistance: the groundwater near the municipal solid waste landfill. Sci. Total Environ. 609, 966–973.
Di, Cesare A., Eckert, E.M., D'Urso, S., Bertoni, R., Gillan, D.C., Wattiez, R., Corno, G., 2016. Co-occurrence of integrase 1, antibiotic and heavy metal resistance genes in municipal wastewater treatment plants. Water Res. 94, 208–214.
Duan, M.L., Gu, J., Wang, X.J., Li, Y., Zhang, R.R., Hu, T., et al., 2019. Factors that affect the occurrence and distribution of antibiotic resistance genes in soils from livestock and poultry farms. Ecotoxicol. Environ. Saf. 180, 114–122.
Eggen, T., Moeder, M., Arukwe, A., 2010. Municipal landfill leachates: a significant source for new and emerging pollutants. Sci. Total Environ. 408 (21), 5147–5157.
Graham, D.W., Olivares-Rieumont, S., Knapp, C.W., Lima, L., Werner, D., Bowen, E., 2011. Antibiotic resistance gene abundances associated with waste discharges to the Almendares River near Havana, Cuba. Environ. Sci. Technol. 45 (2), 418–424.
Ju, F., Li, B., Ma, L., Wang, Y., Huang, D., Zhang, T., 2016. Antibiotic resistance genes and human bacterial pathogens: Co-occurrence, removal, and enrichment in municipal sewage sludge digesters. Water Res. 91, 1–10.
Jutkina, J., Marathe, N.P., Flach, C.F., Larsson, D.G.J., 2018. Antibiotics and common antibacterial biocides stimulate horizontal transfer of resistance at low concentrations. Sci. Total Environ. 616, 172–178.
Kibuule, D., Kagoya, H.R., Godman, B., 2016. Antibiotic use in acute respiratory infections in under-fives in Uganda: findings and implications. Expert Rev. Anti-infe 14 (9), 863–872.
Lemire, J.A., Harrison, J.J., Turner, R.J., 2013. Antimicrobial activity of metals: mechanisms, molecular targets and applications. Nat. Rev. Microbiol. 11 (6), 371–384.
Li, J., Cao, J., Zhu, Y.G., Chen, Q.L., Shen, F., Wu, Y., et al., 2018. Global survey of antibiotic resistance genes in air. Environ. Sci. Technol. 52 (19), 10975–10984.
Li, N., Zhang, X., Wu, W., Zhao, X., 2014. Occurrence, seasonal variation and risk assessment of antibiotics in the reservoirs in North China. Chemosphere 111, 327–335.
Li, Z.Y., Wang, M.Y., Xu, H., 2017. Bacterial class 2 and 3 integrons for the dissemination antimicrobial resistance. Chin. J. Antibiot. 1–7.
Liu, W.J., Long, Y.Y., Fang, Y., Ying, L.Y., Shen, D.S., 2018. A novel aerobic sulfate reduction process in landfill mineralized refuse. Sci. Total Environ. 637, 174–181.
Long, Y.Y., Shen, D.S., Wang, H.T., Lu, W.J., Zhao, Y., 2011. Heavy metal source analysis in municipal solid waste (MSW): case study on Cu and Zn. J. Hazard Mater. 186 (2–3), 1082–1087.
Lu, R., 2000. Analytical Methods of Soil and Agricultural Chemistry. China Agriculture Science and Technology Press, Beijing.
Luo, Y., Xu, L., Rysz, M., Wang, Y., Zhang, H., Alvarez, P.J., 2011. Occurrence and transport of tetracycline, sulfonamide, quinolone, and macrolide antibiotics in the Haihe River Basin, China. Environ. Sci. Technol. 45 (5), 1827–1833.
Luo, Y., Yang, F.X., Mathieu, J., Mao, D.P., Wang, Q., Alvarez, P.J.J., 2014a. Proliferation of multidrug-resistant New Delhi metallo-beta-lactamase genes in municipal wastewater treatment plants in northern China. Environ. Sci. Technol. Lett. 1 (1), 26–30.
Luo, Y., Wang, Q., Lu, Q., Mu, Q.H., Mao, D.Q., 2014b. An ionic liquid facilitates the proliferation of antibiotic resistance genes mediated by class I integrons. Environ. Sci. Technol. Lett. 1 (5), 266–270.
Munir, M., Wong, K., Xagoraraki, I., 2011. Release of antibiotic resistant bacteria and genes in the effluent and biosolids of five wastewater utilities in Michigan. Water Res. 45 (2), 681–693.
Ohore, O.E., Addo, F.G., Zhang, S.H., Han, N.N., Anim-Larbi, K., 2019. Distribution and relationship between antimicrobial resistance genes and heavy metals in surface

sediments of Taihu Lake, China. J. Environ. Sci-China. 77, 323–335.
Price, L.B., Koch, B.J., Hungate, B.A., 2015. Ominous projections for global antibiotic use in food-animal production. Proc. Natl. Acad. Sci. U.S.A. 112 (8), 5554–5555.
Release, N., 2017. WHO publishes list of bacteria for which new antibiotics are urgently needed. Neurosciences 38 (4), 444–445.
Schmittgen, T.D., Livak, K.J., 2008. Analyzing real-time PCR data by the comparative C-T method. Nat. Protoc. 3 (6), 1101–1108.
Shao, S.C., Hu, Y.Y., Cheng, J.H., Chen, Y.C., 2018. Research progress on distribution, migration, transformation of antibiotics and antibiotic resistance genes (ARGs) in aquatic environment. Crit. Rev. Biotechnol. 38 (8), 1195–1208.
Song, L., Gu, D.G., Huang, M.S., Chen, L., Huang, Q.C., He, Y., 2012. Spatial distribution and contamination assessments of heavy metals in sediments of Wenzhou River network. Spectrosc. Spectr. Anal. 32 (9), 2540–2545.
Song, L.Y., Li, L., Yang, S., Lan, J.W., He, H.J., McElmurry, S.P., et al., 2016. Sulfamethoxazole, tetracycline and oxytetracycline and related antibiotic resistance genes in a large-scale landfill, China. Sci. Total Environ. 551, 9–15.
Staley, B.F., de los Reyes, F.L., Barlaz, M.A., 2012. Comparison of Bacteria and Archaea communities in municipal solid waste, individual refuse components, and leachate. Fems. Microbiolo. Ecol. 79 (2), 465–473.
Su, Y.L., Wang, J.X., Xia, H.P., Xie, B., 2019. Comparative network analysis revealing the mechanisms of antibiotic resistance genes removal by leachate recirculation under different hydraulic loadings. Sci. Total Environ. 649, 318–326.
Sun, M.M., Ye, M., Schwab, A.P., Li, X., Wan, J.Z., Wei, Z., et al., 2016. Human migration activities drive the fluctuation of ARGs: case study of landfills in Nanjing, eastern China. J. Hazard Mater. 315, 93–101.
Szabo, L., Steinhardt, M., Homlok, R., Kovacs, K., Illes, E., Kisko, G., et al., 2017. A microbiological assay for assessing the applicability of advanced oxidation processes for eliminating the sublethal effects of antibiotics on selection of resistant bacteria. Environ. Sci. Technol. Lett. 4 (6), 251–255.
Tang, X.J., Shen, C.F., Chen, L., Xiao, X., Wu, J.Y., Khan, M.I., et al., 2010. Inorganic and organic pollution in agricultural soil from an emerging e-waste recycling town in Taizhou area, China. J. Soils Sediments 10 (5), 895–906.
Tang, H.M., Zhang, Y.Y., Hu, J.M., Li, Y., Li, N., Wang, M.Z., 2019. Mixture of different *Pseudomonas aeruginosa* SD-1 strains in the efficient bioaugmentation for synthetic livestock wastewater treatment. Chemosphere 237, 124455.
Threedeach, S., Chiemchaisri, W., Watanabe, T., Chiemchaisri, C., Honda, R., Yamamoto, K., 2012. Antibiotic resistance of Escherichia coli in leachates from municipal solid waste landfills: comparison between semi-aerobic and anaerobic operations. Bioresour. Technol. 113, 253–258.
Wang, F.H., Qiao, M., Su, J.Q., Chen, Z., Zhou, X., Zhu, Y.G., 2014. High throughput profiling of antibiotic resistance genes in urban park soils with reclaimed water irrigation. Environ. Sci. Technol. 48 (16), 9079–9085.
Wang, M.Z., Lai, B.M., Dandekar, A.A., Yang, Y.S., Li, N., Yin, J., et al., 2017. Nitrogen source stabilization of quorum sensing in the *Pseudomonas aeruginosa* bioaugmentation strain SD-1. Appl. Environ. Microbiol. 83 (16) e00870-17.
Wang, W.H., Zhang, W.F., Liang, H., Gao, D., 2019. Occurrence and fate of typical antibiotics in wastewater treatment plants in Harbin, North-east China. Front. Environ. Sci. Eng. 13 (3), 2095–2201.
Wang, Y.Q., Tang, W., Qiao, J., Song, L.Y., 2015. Occurrence and prevalence of antibiotic resistance in landfill leachate. Environ. Sci. Pollut. Res. 22 (16), 12525–12533.
Wu, D., Huang, Z.T., Yang, K., Graham, D., Xie, B., 2015. Relationships between antibiotics and antibiotic resistance gene levels in municipal solid waste leachates in Shanghai, China. Environ. Sci. Technol. 49 (7), 4122–4128.
Wu, D., Huang, X.H., Sun, J.Z., Graham, D.W., Xie, B., 2017. Antibiotic resistance genes and associated microbial community conditions in aging landfill systems. Environ. Sci. Technol. 51 (21), 12859–12867.
Wu, D., Ma, R.Q., Wei, H.W., Yang, K., Xie, B., 2018. Simulated discharge of treated landfill leachates reveals a fueled development of antibiotic resistance in receiving tidal river. Environ. Int. 114, 143–151.
Yang, Y., Li, B., Zou, S.C., Fang, H.H.P., Zhang, T., 2014. Fate of antibiotic resistance genes in sewage treatment plant revealed by metagenomic approach. Water Res. 62, 97–106.
Ying, L.Y., Long, Y.Y., Yao, L.H., Liu, W.J., Hu, L.F., Shen, D.S., et al., 2019. Sulfate reduction at micro-aerobic solid-liquid interface in landfill. Sci. Total Environ. 667, 545–551.
You, X.X., Wu, D., Wei, H.W., Xie, B., Lu, J., 2018. Fluoroquinolones and beta-lactam antibiotics and antibiotic resistance genes in autumn leachates of seven major municipal solid waste landfills in China. Environ. Int. 113, 162–169.
Yu, Z., He, P.J., Shao, L.M., Zhang, H., Lu, F., 2016. Co-occurrence of mobile genetic elements and antibiotic resistance genes in municipal solid waste landfill leachates: a preliminary insight into the role of landfill age. Water Res. 106, 583–592.
Zhang, K., Xin, R., Zhao, Z., Ma, Y.Z., Zhang, Y., Niu, Z.G., 2020. Antibiotic resistance genes in drinking water of China: occurrence, distribution and influencing factors. Ecotoxicol. Environ. Saf. 188, 109837.
Zhang, M.Q., Zhang, M.H., 2007. Assessing the impact of leather industries on the quality of water discharged into the East China Sea from Wenzhou Watersheds. J. Environ. Manag. 85 (2), 393–403.
Zhang, Q.Q., Ying, G.G., Pan, C.G., Liu, Y.S., Zhao, J.L., 2015a. Comprehensive evaluation of antibiotics emission and fate in the river basins of China: source analysis, multimedia modeling, and linkage to bacterial resistance. Environ. Sci. Technol. 49 (11), 6772–6782.
Zhang, R.J., Zhang, G., Zheng, Q., Tang, J.H., Chen, Y.J., Xu, W.H., et al., 2012. Occurrence and risks of antibiotics in the Laizhou Bay, China: impacts of river discharge. Ecotoxicol. Environ. Saf. 80, 208–215.
Zhang, S.H., Gu, J., Wang, C., Wang, P.F., Jiao, S.J., He, Z.L., et al., 2015b. Characterization of antibiotics and antibiotic resistance genes on an ecological farm system. J. Chem. 2015 1–8.
Zhang, X.H., Xu, Y.B., He, X.L., Huang, L., Ling, J.Y., Zheng, L., et al., 2016. Occurrence of antibiotic resistance genes in landfill leachate treatment plant and its effluent-receiving soil and surface water. Environ. Pollut. 218, 1255–1261.
Zhang, Y., Gu, A.Z., Cen, T.Y., Li, X.Y., He, M., Li, D., et al., 2018. Sub-inhibitory concentrations of heavy metals facilitate the horizontal transfer of plasmid-mediated antibiotic resistance genes in water environment. Environ. Pollut. 237, 74–82.
Zhao, R.X., Feng, J., Yin, X.L., Liu, J., Fu, W.J., Berendonk, T.U., et al., 2018. Antibiotic resistome in landfill leachate from different cities of China deciphered by metagenomic analysis. Water Res. 134, 126–139.
Zhou, L.J., Ying, G.G., Zhang, R.Q., Liu, S., Lai, H.J., Chen, Z.F., et al., 2013. Use patterns, excretion masses and contamination profiles of antibiotics in a typical swine farm, south China. Environ. Sci-Proc. Imp. 15 (4), 802–813.
Zhou, Z.C., Feng, W.Q., Han, Y., Zheng, J., Chen, T., Wei, Y.Y., et al., 2018. Prevalence and transmission of antibiotic resistance and microbiota between humans and water environments. Environ. Int. 121, 1155–1161.
Zhu, Y.G., Johnson, T.A., Su, J.Q., Qiao, M., Guo, G.X., Stedtfeld, R.D., et al., 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proc. Natl. Acad. Sci. U.S.A. 110 (9), 3435–3440.