Uncover landfilled antimicrobial resistance: a critical review of antibiotics flux, resistome dynamics and risk assessment

Dong Wu1,2,3,4,#, Yinglong Su1,2,#, Panliang Wang1, Jue Zhao4, Jiawen Xie4 & Bing Xie1,2,*

1Shanghai Engineering Research Center of Biotransformation of Organic Solid Waste, School of Ecological and Environmental Sciences, Shanghai Key Lab for Urban Ecological Processes and Eco-Restoration, East China Normal University, Shanghai 200241, China; 2Shanghai Institute of Pollution Control and Ecological Security, Shanghai 200092, China; 3Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education, Guizhou Medical University, Guizhou 550001, China; 4Department of Civil and Environmental Engineering and Research Institute for Sustainable Urban Development, The Hong Kong Polytechnic University, Hong Kong 999077, China

#Contributed equally to this work.
*Corresponding author (email: bxie@des.ecnu.edu.cn)

Received 1 August 2021; Revised 12 December 2021; Accepted 17 January 2022; Published online 15 July 2022

Abstract: Municipal solid waste (MSW) landfill is one of the most important reservoirs of antimicrobial resistance (AMR) in urban environments. By reviewing ~120 published cases worldwide, we found that leachate-borne antibiotics were at the μg/L level, and meanwhile, around 8 tons of antibiotics (including the clinically relevant ones) annually leached from the MSW landfills in China. During a decade-long landfilling process, the leachate-borne bacteria mainly originating from human-associated waste (>40%) formed a community network being versatile to the drastic environmental changes. Among them, the keystone species (Proteobacteria subtaxa) functioned for metabolizing the most available substrate in leachates and were also the hosts of mobile antibiotic resistance genes (ARGs), which suggested the enduring and close associations between bacterial community and resistome. These leachate-borne ARGs were highly mobile via plasmid-mediated horizontal gene transfer, especially in less aged leachates (<10 yr). MetaCompare showed that the AMR-hazard index of landfill-specific airborne particles (index=20.5) was significantly higher than that of drinking water (index=17.81, P<0.01). Human daily exposure of ARGs amounted to an inhalation of (5.83±0.16)×10^5 copies of ARGs, being tenfold higher than that ingestion of drinking water, which implies landfills as a non-ignorable AMR source.

Keywords: landfill leachates, antibiotic resistance, antibiotics flux, health risks

Introduction

Around 1000 tons of municipal solid waste (MSW) is generated every second on the earth, 50%–80% of which is transferred to landfills [1]. Therefore, landfills are generally considered as the largest reservoir for the disposal of anthropogenic waste. According to the most updated reports, China and the United States of America (USA) are two countries generating the most of MSW on our planet (200–250 vs. 250–290 million tons/year). European countries, as an aggregate (EU-28) annually generates more than 240 million tons of
MSW (20%–30% of landfilling rate), which is at least four times more than that of India (<50 million tons/year). It is noteworthy that MSW contains a series of heterogeneous materials whose physicochemical characteristics vary substantially along with the progress of landfilling [2]. Human daily activities, especially the discharge of products containing antibacterial agents and expired medicines, are the significant contributors to transporting antibiotics from MSW to the sanitary landfills (Figure 1). In the MSW-unregulated areas, landfills are the receptors of waste discharged from clinical settings and pharmaceutical and husbandry industries [3], and these wastes usually harbor more concentrated antibiotics [4]. The clinical-use antibiotics may be well-regulated in developed countries, but the disposal of household-use ones is usually neglected [5,6]. As a result, the landfilled MSW still contains expired medicines, used diapers, and toilet papers [7]. These antibiotic residuals enable the selection of antibiotic resistance (AMR) in the long-term, and the enrichment of antibiotic resistance genes (ARGs) has been documented during a 20-year long MSW landfilling process [8].

Releasing of AMR materials (ARGs, antibiotic resistant bacteria, and antibiotics) via leachate or aero-solization from landfills is an important (Figure 1), but a less apparent gateway for the spread of ARGs [9–12], which could deteriorate the effectiveness of human-use/clinical antibiotics and jeopardize public health. In Europe and the USA, the death toll related to the AMR is ~50,000 per year [13,14]. Although the recorded dosing rates of antibiotics in developing countries are relatively lower (<15 DID; Table 1) [15–17], the antibiotics sale amount in these countries is 2–3 times higher than the ones in the developed countries [18]. The estimated non-prescription use of antibiotics accounted for 36% and 18% of the total use in China and India (Table 1), respectively. The loose surveillance of antibiotic usages and inappropriate disposal of MSW in the third world aggravate antimicrobial resistance (AMR) risks to the health of 64 million people living on our planet [6,19,20]. The accelerated occurrences of emerging AMR in ambient environments in developing countries, such as the polymyxin/colistin resistance (MCR-1) in China [21] and extended-spectrum β-lactamases (NDM-1) in India [22], suggested that more attention shall be paid toward the AMR factors outside the clinical settings [23].

Notably, landfills functioning as the largest reservoirs of anthropogenic waste have not been holistically studied pertinent to its containing AMR issues. The knowledge gaps, including the distributions of antibiotics and resistome during the decade-long landfilling process, dynamic associations between ARGs and the hosting bacterial community, and transfer of ARGs and AMR risks, have yet to be fulfilled. As such, we attempt to establish the first AMR “standing book” of landfills by collecting the published studies of landfills and related environmental biome and then re-analyzing their concentrations of antibiotics and meta-sequencing data, especially in China. The aims of this study are to (1) elucidate the distribution and emission fluxes of antibiotics from landfills, (2) to provide insight into the dynamics of antibiotic resistomes and associations with bacterial communities along with the decade-long landfilling process, (3) to highlight the pathways and AMR bio-hazards exposed to neighboring environments and human beings. This work intends to serve as a guide for solid waste treatment researchers and management professionals who aim at tackling the MSW-associated AMR risks.

**Methodology**

**Keywords selection and retrieved datasets**

Publications and reports related to MSW landfill sites were searched by using keywords such as “MSW
Figure 1
Sources and pathways of AMR materials circulating between landfills and human environments.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>USA</strong> (landfill)</td>
<td>292.4 million tons (50.0%)</td>
<td>268.7 million tons (52.1%)</td>
<td>263.1 million tons (52.5%)</td>
<td>262.2 million tons (53.8%)</td>
<td>251.1 million tons (52.6%)</td>
<td>253.7 million tons (54.3%)</td>
</tr>
<tr>
<td><strong>Antibiotics use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retail/household sector:</td>
<td>6.8×10^6 SU (2015) [18]; non-prescription use: &lt; 5% [20]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>EU-28</strong> (landfill)</td>
<td>246.9 million tons (23.4%)</td>
<td>246.5 million tons (24.5%)</td>
<td>242.9 million tons (25.8%)</td>
<td>241.6 million tons (26.3%)</td>
<td>240.5 million tons (30.3%)</td>
<td>243.6 million tons (32.4%)</td>
</tr>
<tr>
<td><strong>Antibiotics use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital/clinical admission:</td>
<td>21.6 DID (2010)–24.3 DID (2017) [17]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retail/household sector:</td>
<td>&lt;5×10^9 SU (2015) [18]; non-prescription use: &lt;5% [20]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>China</strong> (landfill)</td>
<td>221.1 million tons (n.a.)</td>
<td>200.8 million tons (n.a.)</td>
<td>248.3 million tons (71.7%)</td>
<td>242.5 million tons (75.2%)</td>
<td>206.1 million tons (86.9%)</td>
<td>197.7 million tons (86.2%)</td>
</tr>
<tr>
<td><strong>Antibiotics use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital/clinical admission:</td>
<td>5.1 DID (2000)–10.8 DID (2015) [15,16]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retail/household sector:</td>
<td>10.0×10^9 SU (2015) [18]; non-prescription use 36% [20]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>India</strong> (landfill)</td>
<td>19.8 million tons (40.1%)</td>
<td>n.a.</td>
<td>48.8 million tons (n.a.)</td>
<td>n.a.</td>
<td>47.6 million tons (n.a.)</td>
<td>n.a.</td>
</tr>
<tr>
<td><strong>Antibiotics use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retail/household sector:</td>
<td>12.9×10^6 SU (2015) [18]; non-prescription use 18% [20]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

landfills”, “antibiotics”, “landfill ARGs/ARGs”, “landfill bacterial community”, “ARGs dissemination”, “metagenomics”, which included the information regarding antibiotics consumption rates, concentrations of antibiotic residuals in landfill samples, and sequencing archives of leachates and related environmental samples. Peer-reviewed journal articles retrieved from Scopus and Web of Science databases were the primary sources, while some web search engines were also performed to obtain the reports and documents released by authorities like World Health Organization (WHO) and other national departments/ministries. In total, there were more than one hundred pieces of studies, reports, and investigations with thousands of original data points retrieved (Supplementary information). Based on these sources, we constructed a dataset of concentrations (mainly from the study in China, Supplementary Table S1; Supplementary information-1) that covered a whole life span of landfill sites, especially in China, detailing the concentrations of leachate-borne antibiotics. In addition, the metagenomic and 16S amplicon sequencing data were also retrieved for re-analyses in this study, and ~250 GB metagenomic data of leachates that cover landfills in more than ten major Chinese metropolises were retrieved from MG-RAST server under accession project ID mgp21084 [24], which included the landfill leachate samples with ages from <3 to >20 yr. The retrieved metagenomics sequencing datasets (mgp21084 from the MG-RAST) were utilized by extracting 16S rRNA gene like reads from the metagenomic files via SortMeRNA (version 2.1b) [25]. Meanwhile, considering that few studies reported the landfill leachates with specific landfilling ages in recent time, other 16S rRNA gene sequencing data of the landfills’ leachates with different ages were collected by the accession number of SRP093020 from the National Center for Biotechnology Information (NCBI, https://www.ncbi.nlm.nih.gov/Traces/study/), which covered the whole lifespan of the landfills in China. The accession numbers of all the retrieved sequencing files were provided in Supplementary information (Table S2 and S3, Supplementary information-1).

Characterization and antibiotic resistome

To obtain the profile of antibiotic resistome released from landfills, the retrieved metagenomics sequences were assembled by using Megahit [26], and the generated contigs were utilized for the prediction of antibiotic resistome by the deep-ARG platform at the long-sequence mode [27]. The abundance of identified ARGs was quantified by Salmon into transcript/ppm-reads [28]. To analyze the horizontal gene transfer (HGT) of ARGs in landfill leachates, PlasFlow was used to map ARGs located on the plasmids-like fragments [29]. The taxonomic information regarding the hosts of ARGs containing contigs was obtained by using the software of Assign-Taxonomy-with-BLAST. MetaCompare was used to assess the potential AMR risks of landfill-related samples as previously described [30]. The intake rates of ARGs via ingestion of tap/drinking water and inhalation of PM$_{2.5}$ were analyzed by utilizing the deepARG short-reads mode. The specific information of metagenomic sequencing data was provided in Table S2 and the details (parameters used in the pipelines) of the bioinformatics were in Supplementary information-2 and -3.

Bacterial network, functions, and source tracking

Raw datasets of the leachate samples with definitive landfill site and landfilling ages information (n=36, ~260 GB, Tables S2 and S3) were retrieved from the NCBI (SRP093020; n=16) and the MG-RAST
QIIME2 was utilized to generate the operational taxonomic units (OTUs) in collected leachate samples (~1.7 GB). In total, 512 OTUs, which had a mean abundance higher than 0.01% and occurred in more than 80% of samples [31], were selected to construct the network. The co-occurrence patterns were initially analyzed according to the Spearman correlation matrix and then were constructed with statistically significant relationships ($P<0.05$, $|R|>0.6$), and the bacterial clusters in the constructed network were aggregated by using the algorithm of greedy-modularity in igraph (v1.2.4). The functions of each module cluster were predicted using the software of PICRUSt2 [32]. The topological characteristics of the constructed networks were calculated by using “qgraph” to evaluate the small-worldness index of the connected nodes. For the smallworld networks (index >1.0), the keystone nodes were selected by using the criteria of high degree and low betweenness, and the kicking-one-out strategy was used to analyze the relative importance of each node to the network by calculating the change of the smallworldness index after the removal of the target node and its connections with others. To analyze the potential origins of leachate-borne bacteria (at the genus level), SourceTracker2 was utilized for a close reference classification, which was merged with the downloaded OTUs feature table of 16S rRNA gene amplicon studies from the EMP (ftp://ftp.microbio.me/emp/release1/otu_tables/closed_ref_greengenes/emp_cr_gg_13_8_subset_5k.biom) [33]. The specific information of 16S rRNA gene sequencing data was provided in Table S3. The pipelines used in this section including the characterization of the predicted microbial functions and source-tracking were detailed in Supplementary information-2.

**Estimation of antibiotic flux in China and statistical analysis**

Systematic management of MSW in China can date back to the 1980s. In the last 30 years, the annual generation amount of MSW increased from 30 (MSW-buried (MSW.b)) to 220 million tons (Table 1), growing by ~7% each year (MSW generation increasing rate (MSW.r)) and the landfilling rate (L.r) generally kept around 70% to 80% [34]. The generation quantities of leachates varied as a function of landfill ages, moisture contents, and local climate, which led to fluxes ranging from 100 to 250 L-leachate/ton-MSW (ranges of MSW to leachate ratio (RL.ratio)) annually [35].

$$\text{Leach (ton)} = \frac{L.r \times \sum_{i=1}^{dur} MSW.b(MT) \times (1 + MSW.r\%)^{dur} \times RL.ratio(250L/ton) \times Ab.con.sum(\mu g/L)}{\mu g/ton(10^{12}/1) \times MT/ton(1/10^6)}. \quad (1)$$

Here, we used the RL.ratio of 230 in China [36] and mean concentrations (Ab.con.sum) among all targeted Chinese landfill sites to estimate their national wide leaching fluxes (eq. (1)), and the superscript dur represented the 30 years, during which China started to systematically landfill its generated MSW.

Prior to conducting the statistical analyses, the data were log-transformed or scaled to improve the sample normality or fitness to specific methods. For the datasets that did not fit normal distributions, non-parametric methods were used. The descriptive analyses of the collected data were performed on Excel 2010 (Microsoft Corp.). To exhibit the tempo-dynamics of antibiotics’ concentrations along the landfilling process, geometric means (GM) were used. Statistical significance was defined at a 95% confidence interval, with a $P$ value of <0.05 (two-tailed). The statistical analyses were performed using R (for windows) 4.0.2 (https://cran.r-project.org/). The usage of statistical methods was explained in detail in Supplementary information-3.
Characteristics and leaching flux of landfilled antibiotics

Concentrations of antibiotics in leachates

As shown in Figure 2A, six commonly used antibiotics, including fluoroquinolones (FQs), macrolides-lincomycin-streptomycin (MLS), sulfonamides (SAs), tetracyclines (TCs), β-lactam (BLs), and chloramphenicol (CPs) were frequently detected in the reviewed worldwide landfill leachates. Based on the reviewed cases, CPs were more commonly reported in the less aged landfill samples (<20 yr, Table S1), with concentrations ranging from 50 to 500 ng/L. By contrast, a recent study showed that chloramphenicol was detected at a level of 12,000 ng/L in the leachates from a mature landfill site in Italy [37]. Likewise, FQs and BLs ranging respectively from 100 to 50,000 ng/L and 10 to 2000 ng/L in total (Figure 2A) were commonly detected in the leachates released from young and intermediate landfills, but exhibited higher mean concentrations in mature ones, particularly in terms of the BLs (One-way ANOVA, \( P<0.01 \)). The major components of the reviewed FQs were ofloxacin, ciprofloxacin and pefloxacin in mature, intermediate, and young landfill leachates (Table S1), respectively, while amoxicillin was the predominant antibiotic in all leachates. The concentrations of BLs varied largely across landfill ages, which indeed appeared to be more related to the locations of samples (Figure 2A). The reviewed BLs antibiotics were rarely reported in Turkey and Niger, which could be attributable to the shortage of data sources and might also imply the limited access to the first tier antibiotics in some developing countries [38], such as cephalosporin (Cefotaxime and Cephalexin) and carbapenem (Table S1).

The other three classes of antibiotics, including MLS and SAs, all exhibited a decreased varying trend of their concentrations from young to mature landfill leachates sampling regions (Figure 2A). The highest contents of MLS (>2.5×10^4 ng/L) and SAs (1.0×10^5 ng/L) were detected in Italian landfill leachates (<10 yr), which were 1.5 orders of magnitude higher than the worldwide average levels and also suggested the deficiency of MSW management in the developed world. Notably, SAs more prevalently occurred than other classes of antibiotics (USA, China, Italy, Singapore), especially in the mature leachates, probably due to the high consumption rates of SAs before the 1980s [37,39]. The TCs varied from 20 to 2000 ng/L in leachates irrespective of the landfill ages (Kruskal-Wallis test, \( P=0.61 \)), and they were averaged at (846.4±766.1) ng/L (Figure 2A).

Antibiotics leaching flux from landfills in China

Figure 2A suggests a long-lasting emission of antibiotics from landfills located in China. Other mature/closed MSW landfills, which had more than 35 years of landfilling ages in Italy and Singapore, still released antibiotics at a concentration of ~1 μg/L-leachate [37,40]. Considering that most of the available data by far came from China (Table S1), this study estimated the leaching flux of antibiotics from domestic (China) landfills. To reduce the discrepancies potentially induced by the landfilling ages, geometric means (GM) of each class of antibiotics (Ab.con) in each landfill site were used [41]. And the geometric means were summed to represent the total concentration of antibiotics in different regions of China (Supplementary information-1). As shown in Figure 2B, the targeted landfills were distributed across 16 mega-cities belonging to 13 regions/provinces in China. Overall, probably for its large population and MSW generation amount (Figure 2B), MSW landfills located in Shanghai City discharged the most concentrated antibiotics in leachates.
Figure 2  (A) Concentrations of targeted antibiotics in landfill leachates (log10-transformed ng/L) on a global scale. Dots in different colors represented the different countries, including China, Italy, Niger, Singapore, Spain, Turkey, and the USA. The targeted landfills were categorized into young (<10 yr), intermediate, and mature (>20 yr) groups. (B) The generation of MSW and total concentrations of antibiotics. The MSW generation amount (million tons/yr) in each region/province of China was proportional to the opacity of blue color; the total concentrations (sum of geometric means (GM)) of the antibiotics in landfill leachates were calculated based on a region/province scale. (C) The emission fluxes of each antibiotics belonging to six major classes were calculated based on a national scale (China).
(−50 μg/L), which was followed by Shaanxi (Xi’an City), Inner Mongolia Autonomous Region, Beijing City, and Hunan (Changsha City) Province. Although Guangdong and Jiangsu provinces generated MSW at a rate higher than 20 million tons per year (Figure 2B), their MSW landfill leachates contained lower concentrations of antibiotics (~10 μg/L). This could be explained by the fact that more alternative MSW treatment technologies, such as incineration other than landfills, were applied in Guangdong (Guangzhou, Shenzhen, Zhuhai City) and Jiangsu (Suzhou and Nanjing City) [2], especially for the treatment of antibiotic-containing MSW (drugs, PPCPs, active sludge, etc.). For example, Taiwan annually generated 6 million tons of MSW [42] and meanwhile practiced a zero-landfilling policy [43], which possibly resulted in the lowest contents of antibiotics in landfill leachates (Figure 2B).

Overall, we estimated that 2 billion tons of MSW have been landfilled in China in the last 30 years (eq. (1)), and the total concentration of target antibiotics (151.9 μg/L) is amounting to 7.5 tons of antibiotics annually leaching out from landfills in China (eq. (1)). Among the antibiotics, FQs primarily including norfloxacin and ofloxacin and MLS mainly comprised of erythromycin had concentrations higher than 2 μg/L (Table S1), which were equivalent to a leaching flux of 3.0 and 2.5 ton antibiotics (Figure 2C). Notably, according to the previous investigation [44], fluoroquinolones (27,300 ton) and macrolides (42,200 ton) were estimated as the most heavily used in China as well. This might reflect the close linkages between antibiotic usage and waste disposal (Figure 2C). The SAs were primarily comprised of sulfamethazine and sulfadiazine, amounting to a total of 1.2 tons of SAs released from landfills. In effect, SAs were not frequently used in the current time [44], and therefore their comparatively higher leaching flux may be ascribed to the hydrophilic property [45], which could make them more easily released with leachates than other antibiotics. The occurrences of clinically relevant antibiotics that are mainly used in clinical settings and their usages need prescriptions [46], such as cephalosporin and amoxicillin, reinforced the notion of the input of clinically relevant waste into MSW (Table S1). These β-lactam antibiotics leached at comparatively lower concentrations of 0.5 μg/L (Figure 2A), which nevertheless still contributed to a ~0.1 tons (β-lactam) of emission from landfills per year (Figure 2C).

**Enrichment of ARGs is an evolving event of the microbial community**

*Few significant correlations between antibiotics and ARGs*

As shown in Figure 2B, the contents of antibiotics were detected at the level of micro-gram per liter. Bacteria being long-term exposed to sub-MICs of antibiotics exhibited a stepwise increase of inhibitive concentrations (ng-μg/L) of antibiotics [47]. From this perspective, AMR bacteria can be readily derived from their decade-long incubation with leachates. However, on-site investigations showed the abundances of AMR-related genes were rarely proportional to the contents of antibiotics [48], which seemed to impose negligible impacts on the distribution of ARGs [8]. Song et al. [49] showed that the target ARGs like tetQ and sulI were attenuated in abundance as landfill aging, during which the contents of antibiotics were not correspondingly decreased. Similarly, significant correlations between sulfonamides resistance genes (sulR) and their parallel antibiotics were not observed in landfill leachates [39]. On the one hand, the availability of antibiotic residuals may also affect the relationships between the concentrations of antibiotics and ARGs [50,51]. However, with the addition of antibiotics in MSW [52], ARGs were not significantly enriched during the
landfilling process. On the other hand, most of the reviewed studies utilized the qPCR-based quantification methods, which may not present the full profile of antibiotic resistomes and thereby obtain biased observations. But notably, Zhao et al. [24] showed that concentrations of fluoroquinolones and tetracyclines were negatively correlated with the total ARG abundance, while other antibiotic classes had no significant correlations with the abundance of their corresponding ARGs.

**Compositions of antibiotic resistome released from landfills**

As shown in Figure 3A, there was a cocktail of concentrated ARGs in the resistome released from landfill leachates. As the most abundant AMR type, the mean abundance of multidrug resistance genes ($1.9 \times 10^4$ ppm) was 2 orders of magnitudes higher than domestic wastewater [53]. Other ARGs encoding resistance to macrolide-lincosamide-streptogramin (MLS), aminoglycoside, sulfonamides, tetracycline, and (glycol)peptide/lincomycin also commonly occurred in leachates (Figure 3A). However, only the (glycol)peptide resistant genes, such as \textit{vanS} and \textit{InuD}, were mainly detected in the young landfill leachates (Figure 3B). By contrast, ARGs encoding resistance to sulfonamide were detected as the most prevalent during the whole landfill stages, where the \textit{sul2} was substantially more abundant than its counterparts (\textit{sul1} and \textit{sul3}, Figure 3B). The occurrences of some clinically relevant ARGs, such as \textit{bla}$^{\textit{OXA}}$ (β-lactam resistant, 0.12% of the whole resistome) and \textit{rpoB2} (rifamycin resistant, 0.08% of the whole resistome), suggested that the reviewed landfill waste was the frontline antibiotics and clinical sources and may imply landfill leaching as an important pathway of clinically important AMR. This further echoes the necessity to construct a holistic AMR surveillance system encompassing both medical and environmental realms [23].

**Importance of leachate-borne bacterial community structure to resistome**

Along with the landfilling process, the BOD$_5$ to TN ratio of leachates decreased drastically from $>5$ to 0 and the containing organic carbon became increasingly more recalcitrant (BOD$_5$/COD=0; NH$_4^+$-N/TN $< 2$ in aged leachates) [19]. The drastic changes in environmental conditions reportedly brought about changes in the structure and functions of the bacterial community, which could further influence the compositions of antibiotic resistome [54]. Although the previous study has documented significant correlations between the compositions of bacterial community and ARGs in landfills [8], the importance of community structure and the influence of specific bacterial taxa in determining the correlations have yet to be clearly presented. Overall, as shown in Figure 4A, there were 512 bacterial OTUs included in the small-world network of landfill leachates (Smallworldness index $>3.0$, Supplementary information-2). Here, the small-world network refers to an ensemble in which the mean geodesic (i.e., shortest-path) distance between nodes (OTU) increases sufficiently slowly as a function of the number of nodes in the network [55] and suggests that the bacterial taxa interacted efficiently and closely in the leachate-borne community. The enduring interactions among bacteria, regardless of landfill ages (Figure 4A), have been proven to magnify the dissemination chances of ARGs [54], and may explain the increased abundance of ARGs during the decade-long landfilling process (Figure 3B). Additionally, 72 OTUs were identified as the keystone taxa given their high connection degrees and low betweenness centrality in the network (Figure 4B) [56]. Most of keystone species were classified as \textit{Proteobacterial} subtaxon, which were the predominant components regarding the substrate
Figure 3  (A) Re-analyzed abundance of antibiotic resistome in landfill leachate. The circle dots represented the outliers in the datasets (transcript per million reads, TPM). The colored and black squares indicated the data points and their mean values, respectively. (B) Heatmap of core antibiotic resistome, which contained the ARG subtypes that were detected in more than 50% of the sequencing samples ($n=20$). The relative abundance of core ARGs higher than $10^5$-TPM was annotated with asterisks.
Figure 4 (A) The co-occurrence network interactions of bacteria released from landfill sites \((n=20; \text{Spearman's } |R|>0.6, P<0.05)\) correlation. The nodes represented unique OTU in the data sets. The size of each node is proportional to the relative abundance (>0.1% in abundance in all samples); topological characteristics were characterized by igraph package in \(R\). The nodes were networked in an efficient approach, which was identified as the type of “small-world”, which means geodesic (i.e., shortest-path) distance between nodes (OTU) increases sufficiently slowly as a function of the number of nodes in the network. (B) In this network, the selection of keystone species in the network was based on the high degree and low betweenness, which was further validated by their impacts on the changes in smallworldness index (>1.0) of the bacterial community in landfill leachates. The removal of keystone nodes (degree >65, betweenness <1000) resulted in significantly more pronounced changes in the bacterial community structure \((P<0.01)\). The raw data were uploaded in NCBI and the accession number was provided in the Supplementary information.
metabolism in all landfill-stage leachates and also the primary ARG-hosting taxa (Figures S1 and S2), suggesting that these keystone bacteria carrying ARGs were pervasively associated with other members in the network, and thereby could, to a large extent, facilitate the dissemination of ARGs in leachates.

**Plasmids and human waste assisted the transfer of ARGs**

The factor influencing distribution pattern of ARGs, besides bacterial community [54], is HGT process driving the dissemination of ARGs at a high rate. Figure S2 shows that more than 40% of ARGs were assigned to no specific bacteria taxa, implying that these ARG-containing contigs were possibly assembled from non-chromosomal DNA, such as integrons and plasmids, which are known as typical MGEs. As shown in Figure 5A, there were 25 types of plasmid-carrying ARGs (or partially) retrieved from the sequencing data of landfill leachates, and these mobile ARGs were more diverse in the young leachates with a landfilling age of <10 years. This is consistent with our analyses that none-taxis assigned ARGs were detected as less abundant in mature landfill leachates (Figure S2), probably because the HGT rate of ARGs was restrained by the limited environmental bio-accessible organics and nutrients in the mature leachates [57]. Notably, these identified ARGs-associated plasmids generally belonged to the phyla of *Proteobacteria* and *Firmicutes* (Table S4), which may be attributable to the limited size of the plasmid hosts database [29], but could also suggest a conserved phylogenetic distribution of the HGT of ARGs in leachates [58].

Additionally, compared to the aged leachates, young ones were characterized with more emerging ARGs encoding resistance to clinically relevant antibiotics, such as *catA*, *blaTEM*, and *aadD1* [8,37], which could be directly related to human usage and ill-managed waste disposal. And the 16S rRNA gene sequencing-based analysis shows that human waste, mainly comprised of feces, predominated over all other potential origins of leachate-borne bacteria (>40%; Figure 5B). It has been proven that human fecal pollution was the most important factor driving the HGT-dissemination of ARGs [59]. As such, we may conclude that landfilled AMR was closely associated with anthropogenic wastes (Figure 5B). However, along with the landfilling process, bacteria are becoming more naturally influenced, especially the aquatic environments like rainwater and surface runoffs [60].

**Exposure pathways and risk assessment**

**Landfill leachate is a critical vector**

Leachate is the major vector transferring pollutants from MSW landfills to the neighboring environments and therefore the treatment of landfill leachate is strictly regulated [61]. However, treatment systems affiliated with MSW landfills are designed to remove organic matters, chemical pollutants, and undesirable microorganisms from landfill leachates (Figure 1). Regarding the removal of landfill ARGs, we did not find a homogenous pattern of their removal efficiencies from the reviewed studies [62]. For example, in landfill bioreactors [63], *sul2*, *tetQ*, *aadA1*, and *blaCTX-M* were reportedly eliminated by two orders of (>95%) magnitude, whereas the abundances of *sulR* and *blaR* genes were only reduced by 30–50% in membrane bioreactors [64]. However, regardless of treatment systems types [65], ARGs [66–68] and ARB [69,70] can be continuously released with the discharge of effluents (Figure 1). Zhang et al. [64] found that the contents
Figure 5  (A) Plasmids and their containing ARGs retrieved from landfill leachate samples by using PlasFlow (contigs without annotated taxa information); the red and blue nodes represented the identified ARGs and plasmids (identity >75%, coverage >75%); the dark yellow nodes denoted the leachate samples with different landfill ages. (B) Percentage of collected landfill leachates genera attributable to various environmental setting origins (%) including air/dust, soil, fresh and saline water biome (water, sediments), animal and human-associated waste (feces, sebum, saliva) concerning three landfill stages by using SourceTracker2.
of ARGs in leachate-receiving soil and water were two orders of magnitude higher than that in non-impacted samples. Moreover, the released ARGs from landfills became more closely associated with MGEs and ARB in leachate-receiving water than in leachates [71], which further increased the risk of AMR in the environment.

Pathogenic micro-organisms originate from numerous sources in MSW [72]. Both the enteric (Salmonella spp. and fecal coliforms) and non-enteric bacterial pathogens (Staphylococcus aureus and Pseudomonas aeruginosa) have been detected in MSW [7,73]. The concentrations of fecal coliforms/streptococci generally ranged about 2×10^8–8×10^8 (organisms g⁻¹ dry weight MSW), most of which was contributed by food waste and diapers [74]. As shown by Grisey et al. [75], enteric bacteria, including S. aureus and Salmonella were transferred from landfills to neighboring groundwater, where the content of fecal coliforms was 200,000 CFU/L (Figure 1). Notably, the fecal bacteria from human wastes are ARG carriers in most cases [76]. The spread of emerging ARGs, like mcr-1 and blaNDM, oftentimes stems from the isolates in human feces, food, and clinical waste [22,77], some of which are landfilled in practice [7]. However, compared to the non-enteric bacterial pathogens (Figure 1), they are unlikely to survive in oligotrophic conditions and this may explain that the attribute values of bacteria originating from human waste decreased from young to aged landfill leachates (Figure S3).

Aquifers, microplastics, and reclaimed parks

Apart from leachates, ARGs in landfilled MSW could also be transferred to neighboring environments via underground aquifers (Figure 1). Chen et al. [11] firstly characterized the distribution of more than 150 ARG subtypes and found that groundwater in the close vicinity of the landfill was closely correlated with MGEs. This suggested an increased HGT potential of ARGs, even though no comparisons regarding the abundance of ARGs were conducted between landfill-impacted and non-impacted groundwater. Moreover, the landfilled plastics could be continuously released in the form of microplastics (MPs) into neighboring environments via leachates [78]. These leachate-borne MPs covered by biofilms that harbored plenty of ARGs may travel from landfill sites to the downstream riverine or marine ecosystems [79], continuously facilitating the dissemination of AMR. Recent studies have shown that the presence of MPs in leachates can not only enrich ARGs but also increase their associations with human pathogens [80], which implies the amplified AMR risks to the MPs-receiving environments.

As shown in Figure 1, the stabilized/mature landfill sites are usually reclaimed to parks with the cover of soil and vegetation [81]. However, previous studies demonstrated that the abundance of ARGs and MGEs in aged refuse was equivalent to that of newly landfilled refuse [8,49]. According to the proposed concept of “One Health”, which emphasizes the interdependence of human and environmental health [82], a reclaimed “landfill park” (such as a golf course) streamlines the propagation of AMR from a segregated engineering site to a more populated human environment, where tourists may have direct contacts with AMR material residuals in the topsoil or emitted airborne particles. However, few studies regarding the ARGs and AMR risks in the reclaimed parks have been conducted yet.

Importance of airborne particles

Airborne particles were loaded with nearly all types of ARGs [83]. They are effective vectors for the
transmission of the AMR materials from landfills, especially from the uncovered working platforms (Figure 1A), to neighboring communities [84]. Notably, fine particulates (PM_{2.5}) are more mobile and inhalable than PM_{10} and thereby they could impose more severe AMR risks on human beings [85], but their risks are usually neglected in previous studies [86]. Recent research clearly showed that ARGs were carried by airborne particles from landfill sites and then were transferred to the downwind residential areas [9]. An adult was estimated to daily inhale \(10^{5} - 10^{8}\) copies of PM_{2.5}-associated ARGs [85]. Here, we collected the metagenomic sequencing data of landfill-specific PM_{2.5} samples for analyses (PRJNA623998). As shown in Figure 6A, the averaged relative AMR risk index value of PM_{2.5} samples was 20.5, being significantly lower than that of leachate samples (index=17.8, \(P<0.001\)); nevertheless, the retrieved PM_{2.5} samples still posed a large portion of ARGs hosted by human pathogens (Figure 6A). In addition, inhalation and ingestion are two major exposure pathways to human beings and we found that the AMR risk index value of PM_{2.5} samples was significantly higher than that of drinking water (index=17.81, \(P<0.01\)). Meanwhile, Figure 6B shows that an adult living nearby the landfill site inhales \((5.83\pm0.16)\times10^{5}\) copies of ARGs in total, most of which conferred resistances to the MLS, rifamycin, aminoglycoside, and bacitracin. The intake rate is estimated to be ten times higher than via ingestion of drinking water (\(P<0.05\), Figure 6B).

**What uncovered**

**Limitations and implications**

To the best of our knowledge, this study collected the most available data concerning antibiotics sequencing data of landfill leachates with ages ranging from <3 to >40 years worldwide, which covered the whole lifespan of a typical landfilling system. These datasets, however, were obtained from seven different countries and most of them originated from China. Thus, leaching fluxes of antibiotics from landfills were only estimated on the national (Chinese) scale with low geographic resolutions. In addition, the strategy to retrieve the 16S rRNA amplicon and metagenomic-sequences focused more on the samples with specific landfilling sites and ages, rather than the number of studies due to the lack of available accession numbers and details of samples' information (deposited in the NCBI) from published papers. As such, raw sequencing datasets concerning microbiome sequencing in landfilling environments were suggested to be uploaded. Considering this undermined significance in our review, a national or global scale study of the dissemination of leachate-borne ARGs and assessment of the related AMR risks therefore have yet to be explored.

Nevertheless, China is the major producer and consumer of antibiotics, so its landfill-AMR issues could snapshot the situation in the developing world, where more than two-thirds of human beings on the earth live. The leaching of tons of antibiotics from landfills located in China suggests urgent control of illegal disposal and fly-tipper of antibiotics-containing material into MSW landfills. The leaching of ARGs and the long-lasting dissemination of the resistome, irrespective of landfilling ages and bacterial community structures, flag the importance of AMR contamination from MSW landfill leachates and airborne particles, which necessitate the holistic removal of AMR materials in MSW landfills.

**Perspectives from “One-Heath” conception**

Widespread AMR is a pervasive global health threat, to which landfills substantially contributed. From the
Figure 6. (a) AMR risk matrix computed by using MetaCompare; the \( X \)- and \( Y \)-axis represented the portions of ARGs-carrying contigs and MGE-ARG both carrying contigs in all assembled contigs. And the \( Z \)-axis indicated the portion of contigs annotated with pathogen genomes as well as ARGs and MGEs. (b) Estimated intake rate of ARGs from landfill-specific airborne PM\(_{2.5}\) particles and drinking water. The risk assessment data and calculations were provided in Supporting Information-3.
perspective of “One Health” [87], MSW landfills creating ideal ecological niches for the development and spread of ARGs from an isolated site to human-exposed environments should be closely monitored, especially on the leachates and associated airborne particles. In addition to the high emission flux of antibiotics, the decade-lasting HGT of the released ARGs is the greatest concern that largely enhances the exchange rate of ARGs between human and natural compartments. To mitigate this risk, efficient management of MSW to prohibit the landfill of antimicrobial drugs and clinical wastes is necessary. Meanwhile, it should be recognized that ARGs harbored in feces-contaminated wastes may decrease in abundance, but the ARG dissemination can hardly be contained in the following treatment. Incineration of this part of MSW, which is now being comprehensively practiced in Shanghai, China [88], could be the most feasible approach to reducing or eliminating the landfill-specific AMR exposures to human environments.

Data availability
Sample information, additional statistics, and original data related to all statistics are provided in Supplementary information. To those who have limited access to NCBI or MG-RAST, please contact the authors via dwu@des.ecnu.edu.cn, who is obligated to provide the raw datasets including the sequencing files and original reviewed data of antibiotics within 3–5 working days via the cloud server.

Acknowledgements
The authors thank the reviewers’ enlightening and constructive comments to improve this manuscript and Dr. William Bahureksa (Colorado State University) for his support in manuscript language revisions.

Funding
This work was financially supported by the National Key Research and Development Program of China (2018YFC1901000), National Natural Science Foundation of China (42107457 and 21577038), Hong Kong Scholar Scheme (XJ2018030), the Fundamental Research Funds for the Central Universities, Shanghai City Sci-Tech Joint Research Project in Yangtze River Delta of Shanghai Municipal Science and Technology Commission (20232420700), and Shanghai Engineering Research Center of Biotransformation of Organic Solid Waste (19DZ2254400).

Author contributions
D.W, Y-L.S and B.X designed the entire study and wrote the manuscript with input from all of the authors; D.W., P-L.W, J-W. X and J.Z executed the data collection and analyses. All of the authors read and approved the final manuscript.

Conflict of interest
The authors declare that there are no conflicts of interest to disclose.

Supplementary information
The supporting information is available online at https://doi.org/10.1360/nso/20220012. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.

References
1 Hoornweg D, Bhada-Tata P. What a Waste: A Global Review of Solid Waste Management. Washington, DC 20433


69 Rizzo L, Manaia C, Merlin C, et al. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and


79 Bank MS, Ok YS, Swarzenski PW. Microplastic’s role in antibiotic resistance. *Science* 2020; **369**: 1315.


84 Lui KH, Jones T, Bérubé K, et al. The effects of particle-induced oxidative damage from exposure to airborne fine particulate matter components in the vicinity of landfill sites on Hong Kong. *Chemosphere* 2019; **230**: 578–586.


