Municipal Solid Waste Treatment System Increases Ambient Airborne Bacteria and Antibiotic Resistance Genes

Linyun Li[†]; Qing Wang[†]; Wenjing Bi[†]; Jie Hou[†]; Yingang Xue[§]; Daqing Mao[‡]; Ranjit Das[†]; Yi Luo[†, *]; Xiangdong Li[#, *]

[†] College of Environmental Science and Engineering, Ministry of Education Key Laboratory of Pollution Processes and Environmental Criteria, Nankai University, Tianjin 300071, China

[#] Department of Civil and Environmental Engineering, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong

[‡] School of Medicine, Nankai University, Tianjin 300071, China

[§] Changzhou Environmental Monitoring Center, Key Laboratory of Environmental Protection of Water Environment Biological Monitoring of Jiangsu Province, Changzhou, China

(Linyun Li and Qing Wang contributed equally to this work.)

*** Corresponding author** Phone: +86 (22) 85358553, E-mail: luoy@nankai.edu.cn

*** Corresponding author** Phone: +852 2766 6041, E-mail: cexdli@polyu.edu.hk

ABSTRACT

Landfill and incineration are the primary disposal practices for municipal solid waste (MSW) and have been considered as the critical reservoir of antibiotic resistance genes (ARGs). However, the possible transmission of ARGs from municipal solid waste treatment system (MSWT system) to ambient air is still unclear. In this study, we collected inside and ambient air samples (PM_{10} and $PM_{2.5}$), and potential source samples (leachate and solid waste) in the MSWT system. The results showed that the MSWT system contributed to the increased ambient airborne bacteria and associated ARGs. Forty-one antibiotic-resistant bacteria (ARB) harboring bla_{TEM-1} were isolated, and the full-length nucleotide sequences of the bla_{TEM-1} gene (harbored by identical *bacillus*) from air (downwind samples) were 100% identical with those in the leachate and solid waste, indicating that the MSWT system was the important source of disperse bacteria and associated ARGs in the ambient air. The daily intake burden (DI) level of ARGs via PM inhalation was comparable with that via ingestion of drinking water, but lower than the DI level via ingestion of raw vegetables. The antibiotic-resistant opportunistic pathogen *Bacillus cereus* was isolated from air, with a relatively high DI level of *Bacillus* via inhalation (10^4-10^6 copies/d) in the MSWT system. This study highlights the key pathway of airborne ARGs to human exposure.

INTRODUCTION

Particulate matter (PM) which is a primary cause of haze has become a severe air pollutant in many urban areas,[1-5] and was also identified as a potential source of human carcinogen by the International Agency for Research on Cancer (IARC) in 2013.[6] Several studies have demonstrated that PM exposure is prominently associated with various cardiopulmonary diseases, such as lower respiratory infections (LRI), chronic obstructive pulmonary disease, and lung cancer.[7-10] PM-associated microbiota have long been deeply concentrated in cattle feed yards,[11] indoor,[12] and outdoor.[13-16] In the last decade, increasing concern has been paid on the antibiotic resistant genes (ARGs) in airborne PM, such as that in cattle feed yards,[11, 17] hospital rooms,[18, 19] wet market,[20] wastewater treatment plants,[21] composting plants,[22] and outdoor environments.[14, 16, 23, 24] The most recent study showed that the bacterial communities from Beijing smog harbored a total of 64 ARG subtypes, which represented much higher richness than soil (39 ARG subtypes) and wastewater (19 ARG subtypes), illustrating that the air could serve as a hotspot for the development and transmission of antibiotic-resistant bacteria (ARB) and their associated ARGs.[25]

It is estimated that solid waste in China totals approximately 210 million tons/year, of which approximately 120 and 85 million tons are transported to landfill and incinerator facilities, respectively.[26] Landfill is an open disposal practice[27] for municipal solid waste (MSW) and has become a potential reservoir for residual antibiotics and their related ARGs.[28-32] Recent studies showed that 11 compounds of antibiotics were detected in landfill waste and leachate with concentrations of 5-250 μg/kg and 10-1000 ng/L, respectively,[31] and 12 ARG subtypes were analyzed in leachates with the relative abundance (normalized by the 16S rRNA gene) of 10^{-7}-10^{-3}, as high as that in the wastewater,[25] illustrating the potential of dissemination for

antibiotic resistance via waste and leachates.[27] In addition to the landfill practice, incineration is another widely used disposal practice for the treatment of MSW.[33, 34] However, to date, no study has been conducted on the airborne ARGs collected from the municipal solid waste treatment system (MSWT system), including transfer station (TS), landfill site (LS), and municipal solid waste incinerator (MI), which may be potentially hazardous to human health, especially in the densely populated megacities. Moreover, bacteria and associated ARGs can be disseminated from the environment to humans through external exposure pathways, namely, through drinking water, food, skin contact, and inhalation.[35-38] In contrast to processed drinking water and food upon their ingestion, inhaled air undergoes virtually no treatment, which would mean that airborne PMs are a unique pathway for the dissemination of environmental bacteria and associated ARGs to humans.

In this study, we investigated the airborne bacterial community, associated ARGs, and culturable ARB in the MSWT system in Changzhou, a city of eastern China. PM (PM_{10} and $PM_{2.5}$), leachate and solid waste samples were collected from the MSWT system including two transfer stations, a landfill site and a municipal solid waste incinerator. Quantitative polymerase chain reaction (qPCR), deep 16S rRNA gene sequencing, and bacterial cultivation were conducted first to gain insight into the impact of common ARGs associated with the MSWT system on the ambient air environment. We further estimated the daily intake burden (DI) of ARGs via the inhalation of PM, which was also compared with the ingestion pathways of drinking water and vegetables. To evaluate the potential human exposure to total airborne bacteria, the daily intake burden of 16S rRNA genes was calculated for respiratory inhalation (DI_{PM}) as well. To our knowledge, this is the first study to quantify airborne ARGs in conjunction with the bacterial community, aiming to identify the contribution of the MSWT system to the

dissemination of airborne ARGs and estimate potential human exposure to bacteria and associated ARGs via inhalation.

MATERIALS AND METHODS

Description of Sampling Sites and Sample Collection

Samples were collected in two transfer stations, a landfill site and a municipal solid waste incinerator, during March 24 to 30, 2016, in Changzhou, China (31°47′N, 119°58′E, Figure 1). PM_{10} and $PM_{2.5}$ samples (1.5 m above ground), leachate and solid waste (0-5 cm) were collected at two transfer stations (TS). PM_{10} and $PM_{2.5}$, along with leachate, and solid waste were sampled at a landfill site (LS), including upwind (1.5 m above ground), waste landfilling area (1.5 m above ground), leachate processing area (1.5 m above ground), and downwind (1.5 m above ground). Additionally, the upwind samples (collected at the location of 150 m upwind relative to the LS) were used as the reference site which is not influenced by the waste disposal operation, and the downwind samples were 800 m away from the LS. Similarly, in the municipal solid waste incinerator site (MI), the sampling sites included the upwind (1.5 m above ground), waste and leachate processing area (1.5 m above ground) and downwind (1.5 m above ground). The upwind samples (collected at the location of 100 m upwind relative to the MI) were used as the reference site, and the downwind samples were 200 m away from the MI. Moreover, the PM_{10} and $PM_{2.5}$ control samples (CK), which are not influenced by the MSWT system were collected as a reference site (1.5 m above ground). More details are given in Figure 1.

PM_{10} and $PM_{2.5}$ samples were collected in triplicate using high volume air samplers (Laoying, Qingdao, China).[39, 40] All PM samples were collected on 90 mm quartz fiber filters (Munktell, Sweden). Detailed performance of the high volume air sampler is provided in Text S2 (Supporting Information). The average flow rate of the

air sampler was adjusted to 100 L/min for 20 h (6:00 a.m. to 2:00 a.m. the next day), resulting in a flow-through of volume approximately 120 m^3. All filters were sterilized by baking in a muffle furnace at 400 °C for 5 h prior to sampling. The net weight of each filter was recorded before and after sampling. The concentrations of PM_{10} and $PM_{2.5}$ at the sampling site were estimated by the net weight of each sampling divided by the 20 h flow-through volume per sampling day. Details of the MSWT system (operating conditions, sampling sites, and geographical coordinates) and the corresponding environmental conditions (air temperature, atmospheric pressure, relative humidity and wind speed) are provided in the Text S1, Table S1, and Table S2 (Supporting Information). All samples were transported to the laboratory on ice, and the isolation of culturable bacteria was performed immediately. Samples were stored at -20 °C before subsequent analysis.

Isolation and identification of antibiotic-resistant bacteria

The PM_{10} samples (downwind samples), leachates, and solid waste from TS, LS and MI were dissolved in phosphate buffered saline (PBS) and plated on Luria-Bertani (LB) agar medium supplemented with three common *β*-lactam antibiotics including ceftazidime (8 mg/L), imipenem (1 mg/L) and ampicillin (100 mg/L).[24, 41, 42] In parallel, the samples were also spread on the surface of LB plates without any antibiotic. All the plates were incubated at 37 °C for 24 h. Furthermore, the isolated strains were identified by 16S rRNA gene sequencing and NCBI blast analysis. The full-length $bla_{\text{TEM-1}}$ sequences were identified from the MSWT system and compared using MEGA7.[43-45] The primers and reaction conditions of the full-length $bla_{\text{TEM-1}}$ gene are provided in the Supporting Information (Table S5).

DNA Extraction

Approximately one-half of the PM_{10} filter and two-thirds of the $PM_{2.5}$ filter from each sample were used for DNA extraction. DNA was extracted using Power Soil DNA Isolation KitTM (MoBio Laboratories, Carlsbad, CA, U.S.) according to the manufacturer's instructions. In brief, the filters were placed in 50 ml centrifuge tubes filled with sterile PBS and pelleted at 4 °C by centrifugation at 200 g for 3 h. After gentle vortexing, the resuspension was filtered through a 0.2 μm Supor 200 PES Membrane Disc Filter (PALL, NY, U.S.). Each of the disc filters was cut into small pieces and loaded into a PowerBead Tube from the kit. Total DNA was extracted from all samples. DNA was obtained from isolated strains using the E.Z.N.A. Bacterial DNA Extraction Kit (OMEGA, U.S.) following the manufacturer's instructions. All DNA samples were stored at -20 °C until further analysis.

Real-time quantitative PCR (qPCR) and 16S rRNA

The qPCR was used to detect and quantify a total of 22 subtypes of ARGs conferring to six different classes of antibiotics such as sulfonamides (*sul*1, *sul*2, and *sul*3), tetracyclines (*tet*A, *tet*M, *tet*Q, *tet*W, and *tet*C), quinolone (*qep*A, *qnr*A, and *qnr*S), aminoglycoside (*aacC*2 and *aacC*3), macrolide (*erm*B and *erm*C), *β*-lactam (bla_{TEM-1}, bla_{OXA-1}, bla_{OXA-2}, bla_{OXA-10}, bla_{AmpC}, bla_{VIM-1}, and bla_{SHV-1}), *int*I 1 and the16S rRNA gene. Quantitative PCR analyses were performed on a Bio-Rad IQ5 instrument (Bio-Rad Company, U.S.), as previously described.[46] The PCR product of each gene was cloned into a pEASY-T1 plasmid (TransGen Biotech, Beijing, China), which was used as a positive control. Negative controls contained all components of the PCR mixture without the DNA template. The list of primers and the qPCR assays are described in the Supporting Information (Text S3 and Table S3).

Bacterial 16S rRNA gene sequencing

The DNA samples were sent to Beijing Biomarker Tech, Ltd. (Beijing, China) for sequencing. The V3-V4 region of the 16S rRNA gene was subjected to high-throughput sequencing with the Illumina MiSeq PE300 platform. PCR amplification of 16S rRNA gene was performed using universal primers 338F (5'-ACTCCTACGGGAGGCAGA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The PCR conditions were as follows: an initial denaturation at 98 °C for 2 min, followed by 30 cycles of 98 °C for 30 s and annealing for 30 s at 50 °C, with a final extension of 72 °C for 5 min. The PCR products were segregated by 2% agarose gel electrophoresis, and approximately 450 bp fragments were purified using Agencourt AMPure XP (Beckman Coulter, Inc., CA, U.S.). All the nucleotide sequence data used in the present study have been deposited in the NCBI SRA database under the project ID PRJNA592425.

Data Analysis

Data analysis was performed using R studio,[47] QIIME,[48] Mothur,[49] Microsoft Excel, Origin, SPSS statistics (19.0) and MEGA7. Studies have demonstrated that the 16S rRNA genes can be applied to estimate the total bacterial abundance and to normalize the selected genes from the total bacterial population.[50] The corrections mentioned were determined by Pearson correlation coefficient with the two-tailed test. To obtain high-quality sequence data, less than 100 bp reads, with one or more ambiguous base-calls (N), or those that had < 90% quality scores > Q20 were excluded using Mothur.[49] After neatening, the high-quality sequences were clustered into operational taxonomical units (OTUs) with QIIME for further analysis.[48] Bipartite network analysis showed the co-shared ARGs among air, leachate and solid waste, which were visualized using Gephi version V 0.9.2.[51]

To estimate the airborne ARGs burden which was released from the solid waste and leachate, the ARGs burden (copies/d) was calculated for the solid waste and leachate in LS and MI, based on the following equations:

$$\text{ARGs burden}_{waste}\ (\text{copies/d}) = \text{concentration of ARGs (copies/g)} \times \text{capacity (g/d)} \quad (1)$$

$$\text{ARGs burden}_{leachate}\ (\text{copies/d}) = \text{concentration of ARGs (copies/ml)} \times \text{capacity (ml/d)} \quad (2)$$

For equation 1, the treated waste per day was defined as the capacity in LS and MI, which were 8.00×10^8 g/d and 3.50×10^8 g/d, respectively (Table S2). For equation 2, the treated leachate (capacity) in LS and MI was 7.00×10^8 ml/d and 3.50×10^8 ml/d, respectively (Table S2).

To assess the relative contribution of inhalation to total human exposure to external ARGs, the daily intake burden (DI) of targeted genes was estimated from the respiratory inhalation (DI_{PM}), and intake from drinking water (DI_{dw}) and vegetables (DI_{ve}), based on the following equations:

$$\text{Gene } DI_{PM}\ (\text{copies/d}) = \text{gene concentration (copies/m}^3) \times \text{inhalation rate (m}^3\text{/d)} \quad (3)$$

$$\text{Gene } DI_{dw}\ (\text{copies/d}) = \text{gene concentration (copies/L)} \times \text{ingestion rate (L/d)} \quad (4)$$

$$\text{Gene } DI_{ve}\ (\text{copies/d}) = \text{gene concentration (copies/g)} \times \text{ingestion rate (g/d)} \quad (5)$$

The concentrations of ARGs in the PM samples were measured in the present study, and the ARGs concentrations in drinking water[52] and vegetables[53, 54] were utilized to calculate the ARGs daily intake, assuming equal DNA extraction efficiencies across various environmental media. Here, we used an average inhaled air rate 20 m^3/d per person, 2 L/d for drinking water and 210 g/d for vegetables intake, respectively.[55] The ingestion rate of vegetables was 2.90 g/kg per day, and the average weight of an adult was 70 kg, as recommended by the USEPA.[55, 56]

RESULTS AND DISCUSSION

Airborne PM, total bacteria and ARGs associated with municipal solid waste treatment system

The concentrations of PM_{10} and $PM_{2.5}$ were 92-268 μg/m^3, and 58-202 μg/m^3 in the MSWT system, respectively (Figure 2A). Meanwhile, the concentrations of 16S rRNA genes (copies/m^3) associated with PMs were in the range of $(9.28\pm0.31)\times10^4$-$(6.29\pm0.11)\times10^5$ copies/m^3 (Figure 2B). Specifically, the MI showed the highest PM concentrations, followed by the LS and the TS ($p<0.05$). A similar trend was observed for the 16S rRNA concentrations, which the 16S rRNA concentrations in MI (from $(2.91\pm0.12)\times10^5$ to $(5.47\pm0.25)\times10^5$ copies/m^3) were comparable with those in LS (from $(2.77\pm0.14)\times10^5$ to $(4.21\pm0.30)\times10^5$ copies/m^3), followed by TS (from $(9.28\pm0.34)\times10^4$ to $(2.07\pm0.16)\times10^5$ copies/m^3). The concentrations of the 16S rRNA gene in the MSWT system (10^5 copies/m^3) were comparable with those in the anthropogenic source composting plants (10^5 copies/m^3),[22] but higher than those in urban areas (10^3-10^4 copies/m^3).[37] In addition, the $PM_{2.5}$ levels in the downwind were 1.23 fold (for LS) and 1.40 fold (for MI) higher than those in the upwind, and the concentrations of the16S rRNA gene associated with $PM_{2.5}$ in the downwind were 1.51 fold (for LS) and 1.88 fold (for MI) higher than those in the upwind. These results indicated that both the LS and MI significantly contributed to the ambient airborne PM, and the total abundance of bacteria.[27, 31]

To investigate the effect of PM-associated ARGs and the *int*I 1 gene on ambient air in the MSWT system, the PM control samples (CK) were compared to those from the transfer station (TS). Both downwind and upwind samples were collected from the landfill site (LSD, LSU) and municipal solid waste incinerator (MD, MU) for comparison (Figure 3). Overall, the *int*I 1 gene and up to 16 PM-associated ARGs were significantly (1-2 orders of magnitude) increased in the downwind samples compared to the upwind samples in TS, LS, and MI ($p<0.05$). Previous studies showed that anthropogenic sources (including feed yards, wastewater treatment plants, and

232 composting plants) have been identified as major sources of ambient airborne bacteria
233 and associated ARGs.[11, 22, 57] These results suggest that the MSWT system as the
234 significant source, might possibly contribute to the increased bacteria and their
235 associated ARGs in the ambient air environment.

236 **Tracking the source of airborne ARGs**

237 The i*nt*I 1 gene and up to 22 ARGs were analyzed among air, leachate, and solid waste
238 in the MSWT system (Figure 4). Among the targeted genes, *int*I 1 and up to 16 ARGs
239 were detected in the air samples. However, a total of 22 targeted genes (*int*I 1 and 21
240 ARGs), and 19 targeted genes (*int*I 1 and 18 ARGs) were detected in leachates and solid
241 waste, respectively. The bipartite network analysis revealed that the *int*I 1 gene and up
242 to 16 ARG subtypes were co-shared among air, leachate and solid waste in the MSWT
243 system (Figure 4). It is worth noting that the high absolute abundance of the clinically
244 relevant *β*-lactam resistance gene $bla_{\text{TEM-1}}$ (10^5 copies/m^3) were ubiquitous in the air
245 samples (Figure S1A), agreeing with a previous study showing that the $bla_{\text{TEM-1}}$ gene
246 predominated in air.[58]

247 To examine the source of airborne ARGs adjacent to MSWT system, the OTUs
248 among air, leachate, and solid waste were analyzed (Figure 5A and 5B). Sankey plots
249 revealed that downwind air OTUs were co-shared with those from upwind air (44%),
250 leachate (11%), and solid waste (28%) in LS (Figure 5A). Similar results were also
251 found for MI, indicating that the OTUs of downwind air were co-shared with those
252 from upwind air (24%), leachate (25%), and solid waste (39%) (Figure 5B). These
253 results suggested that the bacteria were communicated among leachate, solid waste and
254 ambient air in the MSWT system along with the direct emission of bacteria or their
255 aerosolization due to natural winds.[22, 59]

Fifty-one ARBs with β-lactam resistance were isolated from the MSWT system, many of which harbored the resistance gene bla_{TEM-1} (41/51 isolations). To further track the source of those airborne ARGs, the full-length nucleotide sequences of the bla_{TEM-1} gene (match 833 bp) harbored by those identical ARBs from air (upwind and downwind sample), leachate, and solid waste in LS and MI were compared (Figure 5C and Figure 5D). The phylogenetic analysis showed that, in LS, bla_{TEM-1} sequences harbored by *Bacillus megaterium* (from leachate) and *Bacillus sp.* (from solid waste) were clustered with those from downwind air in the same branches (with 100% identity). Similar results were also found in MI that bla_{TEM-1} sequences harbored by *Bacillus subtilis* (from leachate), *Bacillus sp.* and *Bacillus pumilus* (from solid waste) were clustered with those from downwind air in the same branches. These results indicated that the solid waste and leachate in the MSWT system were the important sources of dispersed bacteria and associated ARGs in ambient air. Additionally, *Bacillus* from the source had a high absolute abundance (10^6-10^8 copies/ml of leachate and 10^9 copies/g of solid waste) in the MSWT system.

The class 1 integron is the key mobile genetic elements (MGEs), which can capture ARGs and incorporate them onto their gene cassettes by site-specific recombination. *Int*I 1 is increasingly associated with the dissemination of ARGs, especially in the context of anthropogenic impacts.[60, 61] In this study, *int*I 1 was widely detected in all samples, with an absolute abundance 10^3-10^5 copies/m^3 (Figure S1A). This result was in good agreement with previous studies showing that *int*I 1 was widespread in the atmospheric environment.[37, 58] Additionally, significant positive correlations were observed between the absolute abundance of targeted genes (16S rRNA, *int*I 1, *sul*1, *tet*Q, bla_{TEM-1}, and *erm*B gene) and $PM_{2.5}$ concentrations ($r = 0.87, 0.79, 0.80, 0.76, 0.90, 0.83$, $p<0.01$) (Figure S2). This result was in agreement with a previous study

showing that the high concentration of 16S rRNA genes was due to the high concentration of particulate matter.[22] In contrast, a previous study showed that the abundance of the 16S rRNA gene in ambient air $PM_{2.5}$ samples was not correlated with the concentration of $PM_{2.5}$ in three typical regions.[37] The discrepancy between the two studies is caused by the different sample types. In the present study, we collected the air samples in the MSWT system which is the key source of the $PM_{2.5}$, and continually released PM to the ambient air environments. The previous study collected air samples from the ambient air in urban settings.[37]

Potential human exposure to airborne ARGs

According to the aforementioned results, the leachate and solid waste, as the important sources of dispersed bacteria and associated ARGs in the ambient atmosphere, increased the potential human exposure to airborne ARGs. Although it is difficult to make a concise estimation of the airborne ARGs burden released from the solid waste and leachate, the ARGs burden (copies/d) was calculated for the solid waste and leachate in LS and MI. It was shown that the ARGs burden of solid waste (2.49×10^{21} copies/d) in MI was higher than that (1.62×10^{20} copies/d) in the LS. However, the ARGs burden of leachate (2.42×10^{19} copies/d) in LS was lower than with that of the leachate (1.19×10^{20} copies/d) in MI (Figure 6A). A similar trend was~~were~~ observed in the corresponding air samples, in which the absolute abundance of airborne ARGs from MI (7.51×10^5 copies/m^3 for PM_{10} and 1.69×10^5 copies/m^3 for $PM_{2.5}$) was higher than that from LS1 (1.3×10^5 copies/m^3 for PM_{10} and 1.66×10^5 copies/m^3 for $PM_{2.5}$), and the absolute abundance of airborne ARGs from LS2 (1.74×10^5 copies/m^3 for PM_{10} and 5.37×10^4 copies/m^3 for $PM_{2.5}$) was lower than that from MI. (1.21×10^5 copies/m^3 for PM_{10} and 1.13×10^5 copies/m^3 for $PM_{2.5}$). The similar trend indicated that the absolute abundance of airborne ARGs increased with the increase of ARGs burden of solid waste

306 and leachate. These results suggest that the MSWT system could be regarded as a
307 reservoir of ARGs, which, in turn, may affect the ARGs in the ambient atmosphere
308 environment.[32, 62]

309 The PM-associated ARGs that can be inhaled through the respiratory system may
310 have different fates compared with those of water- and food-borne ARGs in the
311 gastrointestinal tract.[36, 37] It is imperative to compare the daily intake burden of targeted
312 genes via different exposure pathways, to perform a comprehensive analysis of human
313 exposure to targeted genes. The daily intake burden of ARGs from respiratory
314 inhalation (DI_{PM}), drinking water (DI_{dw}) and vegetables (DI_{ve}) was calculated, as shown
315 in Figure 6B. We found that the level of daily intake burden via inhalation of PM was
316 comparable with the ingestion of drinking water, but lower than the ingestion level via
317 raw vegetables. However, taking into account the effects of cooking/boiling on ARGs
318 from raw vegetables and drinking water, the exposure to ARGs through direct PM
319 inhalation without any prior treatment might be even higher. In particular, the daily
320 intake burden of the *β*-lactamase resistance gene bla_{TEM-1} via inhalation (10^6-10^7
321 copies/d) exceeded that via ingestion of drinking water (10^4-10^5 copies/d), which might
322 increase the risk to human health because *β*-lactamase resistance genes threaten the last
323 frontier for antibiotics. Similar results were obtained in a previous study showing that
324 the comparable exposure level for bla_{TEM-1} between drinking water and $PM_{2.5}$ occurred
325 in urban areas.[37]

326 Long-term exposure to PM-associated antibiotic-resistant pathogens increased the
327 potential risk of respiratory tract infection and further caused by cardiopulmonary
328 diseases, which, in turn, may render treatment ineffective due to antibiotic resistance.[7,
329 63, 64] In this study, to evaluate the potential human exposure to airborne total bacteria,
330 the daily intake burden of the 16S rRNA gene was calculated for respiratory inhalation

331 (DI_{PM}) based on Equation 3, and the results were 10^6-10^7 copies/d. Among the total
332 airborne bacteria via inhalation, the antibiotic-resistant opportunistic pathogen *Bacillus*
333 *cereus* was isolated from the air (downwind sample) in the MSWT system (Table S4),
334 with a relatively high DI level of *Bacillus* via inhalation (10^4-10^6 copies/d, based on
335 Equation S1 of Supporting Information). *B. cereus* was reported to be isolated from
336 animal feeding operations (CAFOs)[65, 66] and caused food poisoning,[67] septicemia,
337 surgical wound infection, pneumonia or meningitis in immunocompromised patients.[68]

338 **ENVIRONMENTAL IMPLICATIONS**

339 The present study revealed that the leachate and solid waste in municipal solid waste
340 treatment system are important sources of dispersed bacteria and associated ARGs in
341 the ambient atmosphere environment. According to the estimate of the daily intake
342 burden (DI) of ARGs, the DI level via PM inhalation was comparable with that via
343 intake of drinking water, but lower than the ingestion level via raw vegetables. The
344 antibiotic-resistant opportunistic pathogen *Bacillus cereus* was isolated from air and the
345 DI level of *Bacillus* via inhalation was 10^4-10^6 copies/d. Considering the ARGs
346 exposure burden via inhalation, further studies are critically needed to explore the
347 complex interactions among the airborne microbiome (pathogen, virus, fungi, etc.),
348 associated ARGs and innate pathogens in the human respiratory system, especially
349 airborne ARGs which is located on the mobile genetic elements acquired by human
350 pathogens. This study highlights the route of airborne transmission of ARGs from
351 municipal solid waste treatment system, consequently increased the ARGs exposure
352 risk via inhalation to nearby residents.

ASSOCIATED CONTENTS

Supporting Information

The Supporting Information is available free of charge on the ACS Publications websites.

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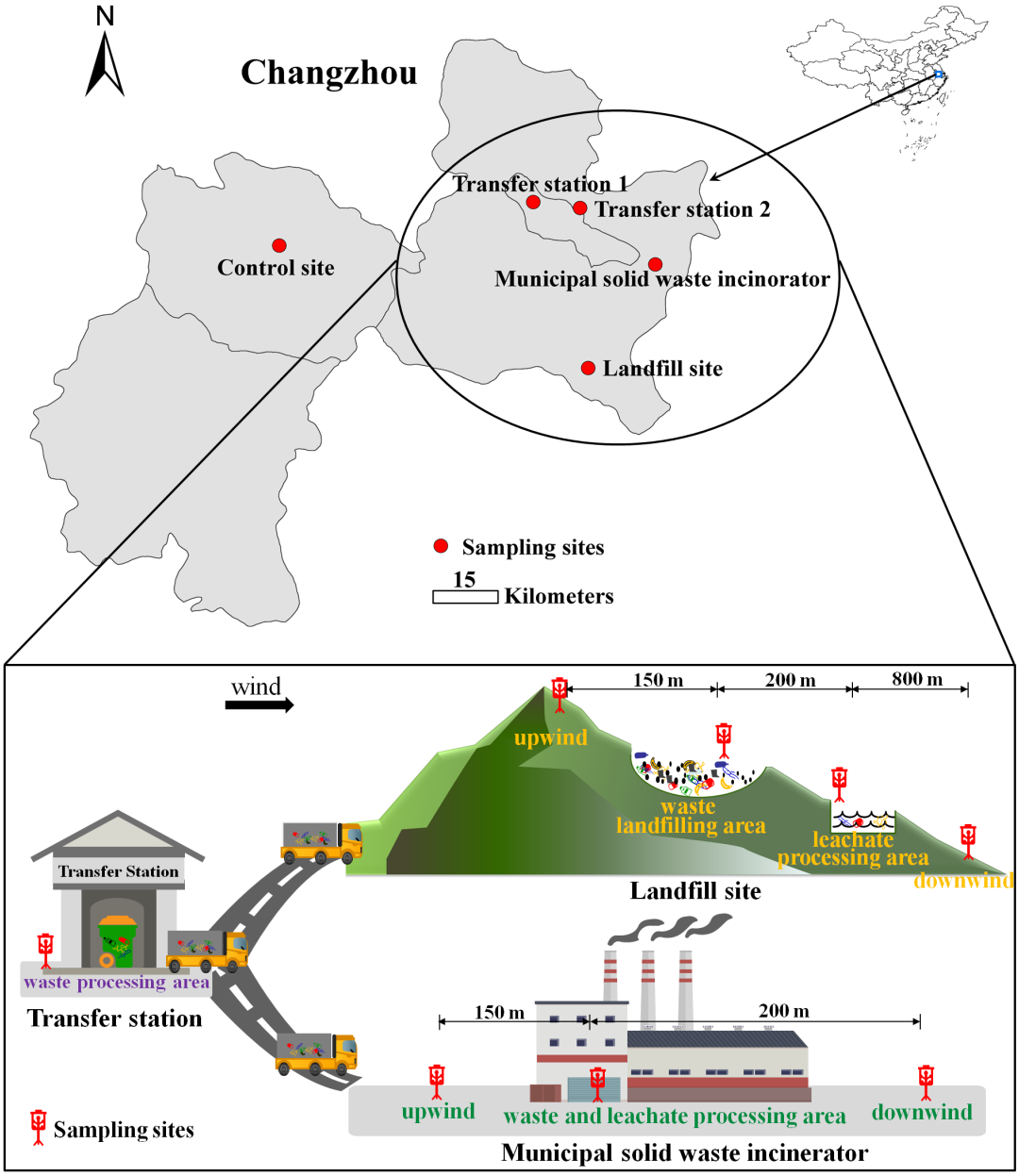
Declarations of interest

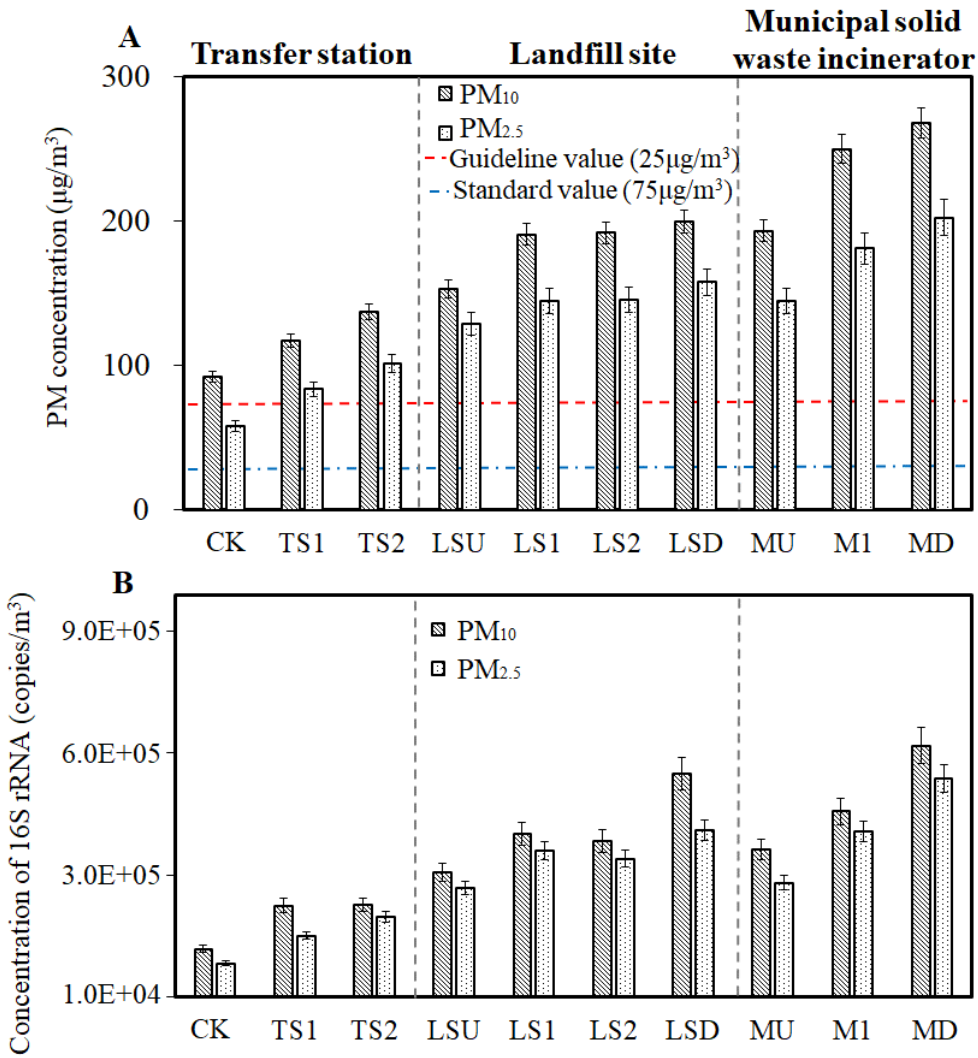
The authors declare no competing financial interests.



368

369 **Figure 1.** Geographical location of sampling sites in transfer station (TS), landfill site (LS) and

370 municipal solid waste incinerator (MI).



371

372 **Figure 2.** The concentrations of PM and 16S rRNA gene. (A) PM (PM_{10} and $PM_{2.5}$) concentrations

373 from all collected samples, (B) Concentrations of 16S rRNA genes from PM_{10} and $PM_{2.5}$ from all

374 collected samples. CK: the PM samples control site; TS1: transfer station 1; TS2: transfer station 2;

375 LSU: landfill site-upwind; LS1: landfill site-leachate processing area; LSD: landfill site-downwind;

376 MU: municipal solid waste incineration-upwind; M1: municipal solid waste incineration-waste and

377 leachate processing area; MD: municipal solid waste incineration-downwind.

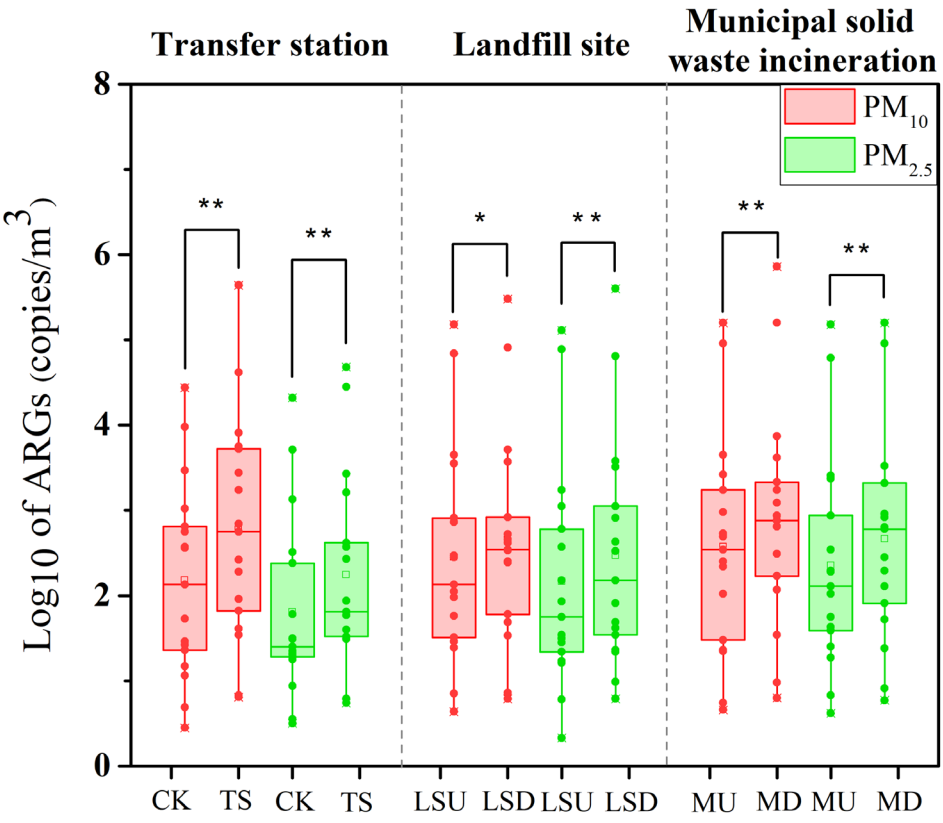
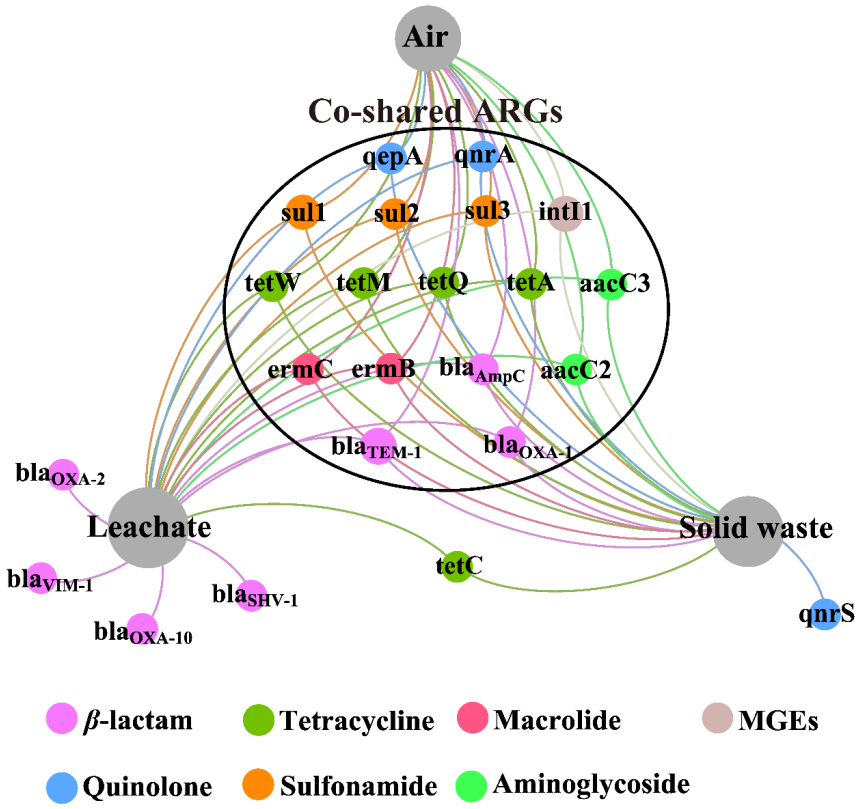


Figure 3. The absolute abundance of *int*I 1 and sixteen ARGs conferring to six classes of antibiotics (PM_{10} and $PM_{2.5}$) in the transfer station, landfill site, and municipal solid waste incinerator. The PM control samples (CK) were compared to those from the transfer station (TS). Both downwind and upwind samples were collected from the landfill site (LSD, LSU) and municipal solid waste incinerator (MD, MU) for comparison (*p<0.05, * *p<0.01). CK: the PM samples control site; TS: transfer station; LSU: landfill site-upwind; LSD: landfill site-downwind; MU: municipal solid waste incineration-upwind; MD: municipal solid waste incineration-downwind.



386

387 **Figure 4.** Bipartite network analysis revealing the co-shared ARGs and MGEs among air, leachate

388 and solid waste in the MSWT system. On the basis of the antibiotics they resist, we classified ARGs

389 into six categories (sulfonamide, tetracycline, quinolone, aminoglycoside, macrolide, *β*-lactam), and

390 the same category was labeled with the same color.

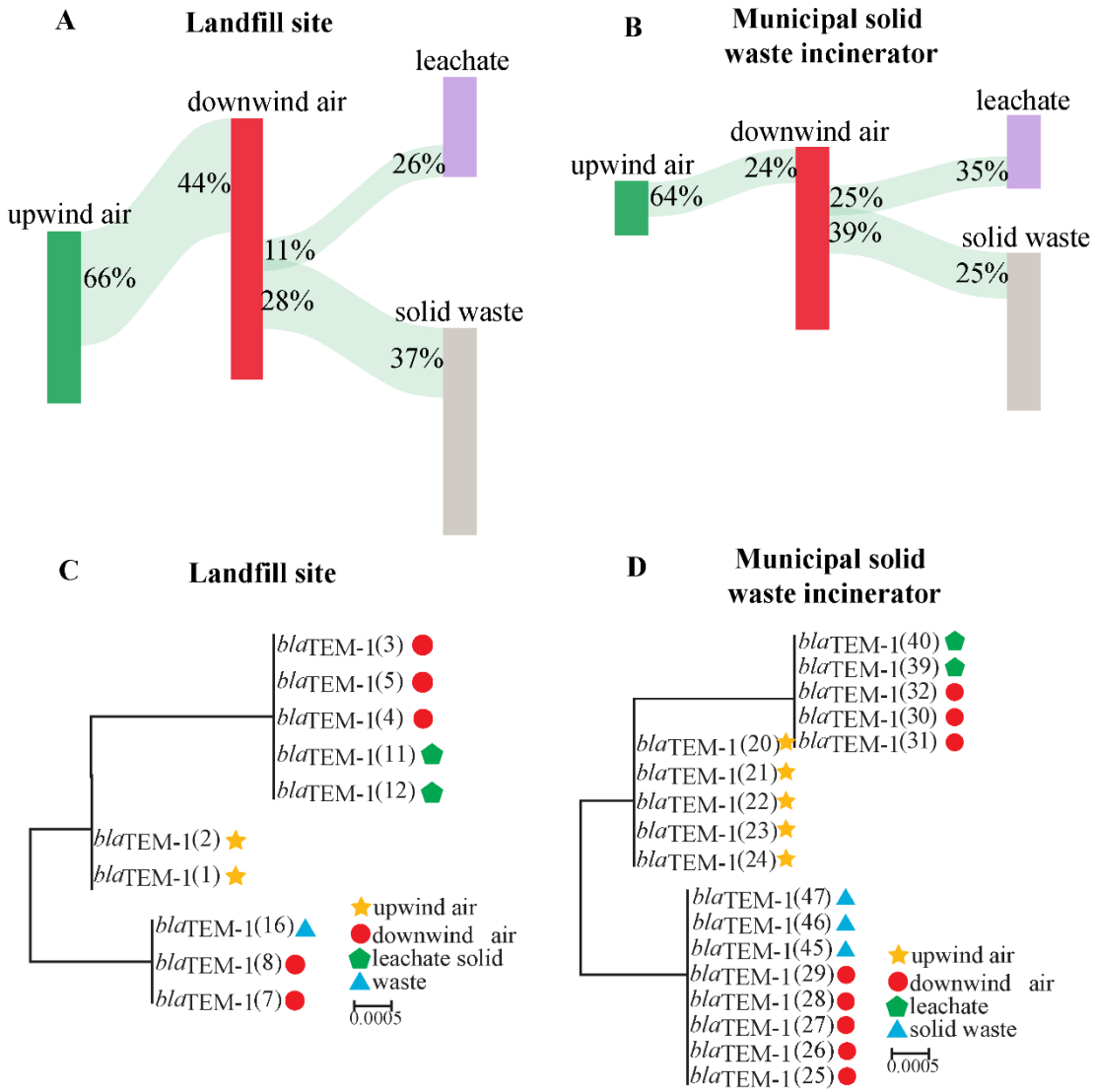
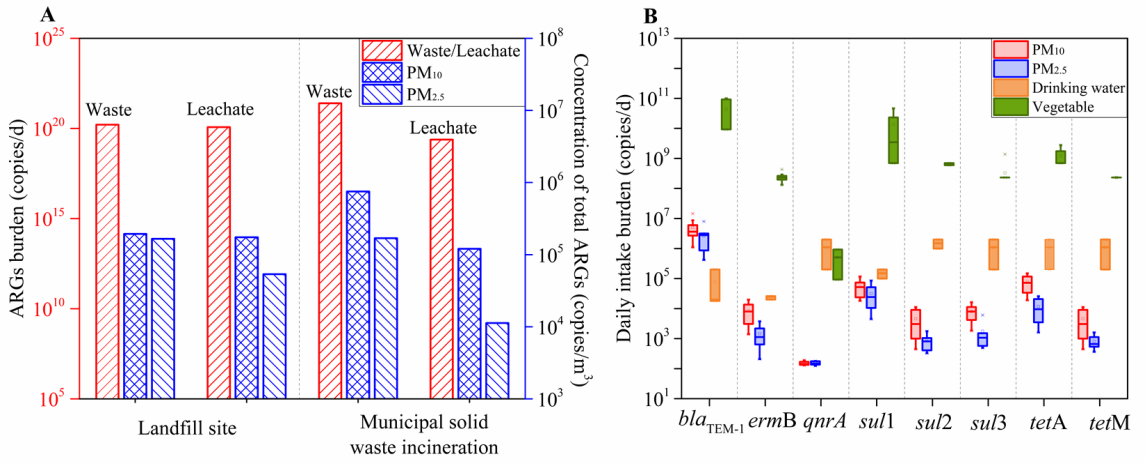


Figure 5. OTUs which are co-shared among air, leachate and solid waste are tracked using Sankey plots in (A) landfill site and (B) municipal solid waste incinerator. The heights of rectangles indicate the relative number of OTUs. The lines represent the transfer of OTUs among air, leachate and solid waste. Phylogenetic analysis on *bla*$_{\text{TEM-1}}$ genes of upwind air, downwind air, leachate, and solid waste in (C) landfill site and (D) municipal solid waste incinerator. The branches of phylogenetic tree contained *bla*$_{\text{TEM-1}}$ genes arising from 10 identical bacteria (isolated in landfill site) and 18 identical bacteria (isolated in municipal solid waste incinerator). The number in brackets correspond

399 to the number in the Table S4. The cut-off value of consensus tree was set to 50% and the scale

400 length was 0.0005.



401

402 **Figure 6.** (A) Compared the ARGs burden of solid waste (copies/d), leachate (copies/d) with local

403 PM_{10} and $PM_{2.5}$ (copies/m^3) in the landfill site and municipal solid waste incinerator. The calculation

404 was based on equation 1-2. (B) Comparison of the potential human daily intake burden of ARGs

405 between inhalation (red for PM_{10} and blue for $PM_{2.5}$) and ingestion (orange for drinking water and

406 green for the vegetables). The calculation was based on equations 3-5.

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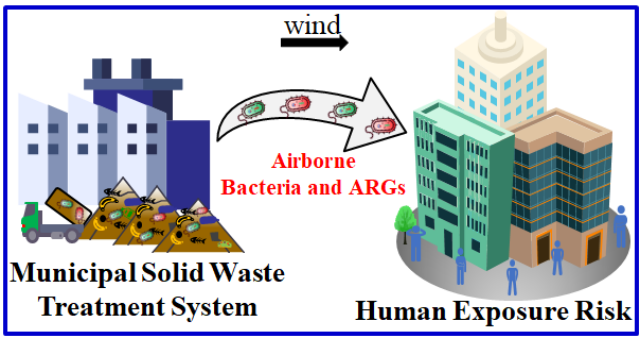

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638 **TOC Art**