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Release of airborne antibiotic resistance genes from municipal solid waste transfer stations

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Abstract

With urbanization, municipal solid waste (MSW) transfer station as an important link in the process of refuse collection and transportation is closer to residents' living areas. At present, studies on airborne antibiotic resistance genes (ARGs) generated during MSW management are still few and not comprehensive enough. In this study, metagenomics and high-throughput sequencing methods were used to analyze the composition and content of bioaerosol and airborne ARGs in the waste reception hall and the top vent of three MSW transfer stations. 265 ARG subtypes were detected. The main subtypes were *bac*A, multidrug_transporter, *mex*W, *sul*2 and *mac*B, and the main types were multidrug resistance genes and bacitracin resistance genes. Different microbes and ARGs showed diverse release characteristics, but in general, the concentration of bacteria and ARGs at the top vent of the transfer station was higher than that in the waste reception hall, and fungi were more sensitive than bacteria when passing through the odor treatment system connecting between the top vent and the waste reception hall. For ARGs, daily intake of a worker was calculated to be 1.08×10^{10}–2.79×10^{10} copies d^{-1} and environmental release was 2.88×10^{9}–9.49×10^{9} copies m^{-3}. Therefore, control measures for airborne ARGs and pathogenic microorganisms are urgently needed to ensure the health of workers and surrounding residents.

Keywords: MSW collection & transportation, Airborne antibiotic resistance genes, Bioaerosol, Daily intake, Metagenomics, High-throughput sequencing

1 Introduction

With the rapid increase of antibiotic use worldwide, antibiotic resistance caused by the wide spread of antibiotic resistance genes (ARGs) has become a serious threat to human health and survival [1]. According to the statistics of World Health Organization in 2019, drug-resistant diseases caused at least 700,000 deaths globally per year, and the number may reach 10 million by 2050 [2]. Many studies have shown that there exist a huge amount of ARGs in the air of human's living regions [3–5], wastewater treatment plant (WWTP) [6] and agricultural farms [7]. As emerging pollutant [8], ARGs can exist in the air for a long time and be transported via aerosols [9]. If ARGs are carried by human pathogens and enter the body through inhalation, they could undermine the effectiveness of antibiotic treatment and endanger public health [10]. However, the research on the airborne ARGs involved in the management (including collection, transportation and treatment) of municipal solid waste (MSW) has not yet been paid enough attention to, although solid waste and its leachates in the MSW treatment facilities have been found to contain a large number of ARGs [11–13].

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As a result, the MSW management system would become an ARG source with increasing exposure risk to residents through biological aerosols [13, 14].

Li et al. [13] used quantitative polymerase chain reaction (qPCR) and deep 16S rRNA gene sequencing to detect the ARGs and bacteria in the samples collected inside and ambient air samples (PM_{10} and $PM_{2.5}$) from two transfer stations, a landfill site, and a MSW incinerator in Changzhou; their results showed that the daily intake of ARGs via PM inhalation in these areas was comparable with that via drinking water. Gao et al. [15] used droplet digital polymerase chain reaction and 16S rRNA gene sequencing to investigate air samples from the packing, office, composting, and downwind areas of four composting plants. However, the composition of ARGs cannot be fully reflected by such PCR techniques because the targeted ARGs are usually limited to only tens of species [12, 16]. Comparatively, metagenomics based on high-throughput sequencing has many advantages [17]. It can provide more comprehensive and accurate information in the study of ARGs. At present, metagenomics has been applied to research on airborne ARGs in different environments, such as public transit [18], hospitals [19], WWTP and animal farms [20]. Compared with solid and liquid samples, the application of metagenomics on airborne aerosols is quite challenging, owing to low DNA quantities that could be collected [21].

Therefore, the present study initiated the study on airborne ARGs from MSW transfer stations using metagenomic method. The main consideration for focusing on transfer stations rather than landfills was that they are usually closer to residential areas. Landfills, as terminal disposal sites for solid waste, are usually more than 500 m away from residential areas [22], while MSW transfer stations may be only 30 m or even closer [23]. Therefore, although transfer stations are typically smaller than landfills, the health risks cannot be ignored. In this study, we collected bioaerosol samples from the waste reception hall and the top vent of three MSW transfer stations in Shanghai. qPCR, 16S and 18S high-throughput sequencing were used to detect microbial communities (bacteria, archaea and fungi) in the bioaerosol samples. Then metagenomic analysis of the airborne ARGs was carried out and the potential health and environmental risks were preliminarily assessed. To our knowledge, this is the first study about the release characteristics of airborne ARGs from MSW transfer stations using metagenomics. It is of great significance for comprehensively understanding the full ARGs spectrum of the bioaerosol released from MSW transfer stations.

2 Materials and methods

2.1 Sampling

The sampling sites were at the waste reception halls and top vents of three MSW transfer stations in Shanghai, China. Information about the MSW transfer stations is shown in Table 1. The three MSW transfer stations were all located downtown, serving Hongkou District (HK), Huangpu District (HP) and Jing'an District (JA) respectively, which are the three districts with the highest population density in Shanghai [24].

The three transfer stations treated 280–640 t d^{-1} waste, including household food waste, restaurant food waste and residual waste. Household food waste and restaurant food waste are both perishable wastes. The former is the leftovers from restaurants, canteens and other catering industries, with the characteristics of large quantity and relatively concentrated production, while the latter mainly refers to the discarded scraps and leftovers from residents' daily cooking, which is large in quantity but relatively dispersed. Residual waste refers to other domestic waste except recyclables, harmful waste and perishable waste.

Flora-derived deodorant spray facilities were installed in all three waste reception halls. Flora-derived deodorant with flora extract as the main active component is now widely used in MSW transfer stations in China. In addition, the three MSW transfer stations were equipped with odor collection and treatment facilities. At HK transfer station, the gas passed through a bag filter and spray scrubbers before being discharged from the top vent. Deodorization equipment in HP transfer station

Table 1 Information of MSW transfer stations

Transfer station	Geographical coordinates	Sampling site	Treated waste (t d^{-1})	Population density (people km^{-2}) [24]
Hongkou Transfer Station	31°16′09″ N 121°28′18″ E	HKR (Waste reception hall) HKO (Top vent)	640	33,816
Huangpu Transfer Station	31°12′40″ N 121°30′11″ E	HPR (Waste reception hall) HPO (Top vent)	600	31,808
Jing'an Solid Waste Transfer Center	31°14′23″ N 121°27′02″ E	JAR (Waste reception hall) JAO (Top vent)	280	28,680

included a bag filter and a photocatalytic oxidation unit. The gas collected at JA transfer station was washed with water before being discharged.

The waste reception hall was the main working area for workers. One side of the hall was equipped with waste dumping ports, and the sampling site was located in the rest area on the opposite side (Fig. S1 of Supplementary Materials). The top vent was 15 m above the ground, and the sampling device was placed directly opposite the vent at a distance of 2 m, because the top vent was usually a monitoring sampling site regarded as air pollution source at this height [25]. The average airflow rate of the top vents of the three transfer stations during working hours was 31,000 (HK), 30,000 (HP) and 13,000 $m^3 h^{-1}$ (JA). Each site was sampled for three times completed in 3 days. Each time two parallel samplers were run simultaneously. Since simply metagenomic sequencing already requires more than 50 ng of DNA, three sets of two parallel samples at each sampling site were mixed for 16S rDNA sequencing, 18S rDNA sequencing, metagenomic sequencing, and qPCR. Sample information is shown in Table S1.

The samples were collected using the KB-120F medium flow sampler (Jingcheng, Qingdao, China). The sampler had the particle separate device (with the 50% cut point diameter, $D_{\alpha 50} = (10 \pm 0.5)$ μm) and trapped particulate matters with aerodynamic diameters of ≤10 μm on the Whatman GF/A quartz fiber filters (1.6 μm pore size, 90 mm diameter). All filters were baked in a muffle furnace at 450 °C for 5 h prior to sampling. After cooling, they were put into a sterile polythene Ziplock bag for later use. All relevant instruments were sterilized with 75% alcohol before use. The sampler was placed at a height of 1.5 m and sampled at a flow rate of 100 ± 3 L min^{-1} for 6 h. A filter was placed in a non-running sampler for 5 min before each sampling as a negative control. After sampling, each filter was taken back to the laboratory in a clean petri dish and stored at 4 °C, protected from light.

2.2 Sample pretreatment and DNA extraction

Sample pretreatment before DNA extraction was modified from the reported method [21]. Briefly, 5 mL sterile 10 × *phosphate buffered saline* and 45 mL sterile water were successively added to the 50 mL centrifuge tube. The filter was then rolled and inserted into the tube, and centrifuged at 200×*g* at 4 °C for 3 h. The microbe suspension was filtered by 0.2 μm polyethersulfone (PES) filter. Then the PES filter was cut into small pieces and put into a PowerBead Tube from the PowerSoil DNA Isolation Kit (Qiagen, Hilden, NRW, Germany) for subsequent DNA extraction operations. DNA extraction was performed according to the Kit instructions. All DNA samples were kept at − 80 °C.

2.3 Microbial diversity of prokaryotes and fungi

Nanodrop 2000C (Thermo Fisher Scientific, Waltham, MA, USA), an ultra-micro ultraviolet spectrophotometer, was used for measuring DNA quality and concentration. DNA integrity was detected by agarose gel electrophoresis with a concentration of 1% at 5 V cm^{-1}. After the DNA was qualified, the prokaryote-specific 16S rDNA primer F515 (5′-GTGCCAGCMGCCGCGGTAA-3′) and R806 (5′-GGACTACVSGGGTATCTAAT-3′) [26] was used for the amplification of V4 region. Meanwhile, eukaryote-specific 18S rDNA primer 817F (5′-TTAGCATGGAAT AATRRAATAGGA-3′) and 1196R (5′-TCTGGACCT GGTGAGTTTCC-3′) [27] was used for the amplification of V5 region. Then the PCR products were subjected to paired-end sequencing (2 × 300 bp) using MiSeq platform (Illumina, San Diego, CA, USA).

2.4 Real-time qPCR

The qPCR performed on Mastercycler® ep realplex2 (Eppendorf, Hamburg, Germany) was used to quantify 16S rDNA and 18S rDNA. A single qPCR system was 25 μL, including 9.5 μL sterile water, 12.5 μL SYBR Green Mix (Takara, Japan), 0.5 μL of each primer (10 μM) [28, 29], and 2 μL standard plasmid or DNA sample. Negative control did not contain DNA templates. The sample group and negative control were performed in triplicate, and the positive control was performed in duplicate. The temperature program was set as follows: 3 min initial denaturation at 95 °C, 40 cycles of 20 s at 94 °C, 30 s at annealing temperature (Table 2) and 30 s at 72 °C, a final extension for 7 min at 72 °C. The result is considered credible when the R^2 value of the standard curve is higher than 0.990 and the amplification efficiency is between 80 and 120%.

Table 2 Primer information of qPCR

Gene	Sequence		Annealing temp (°C)
16S rDNA [28]	FW	CGAATATGGAATCCCTAGTAACT	57.5
	RV	GCCCACTCAGTTCGATACGC	
18S rDNA [29]	FW	CTTGGTCATTTAGAGGAAGTAA	58.0
	RV	GCTGCGTTCTTCATCGATGC	

Note: *FW* Forward, *RV* Reverse

2.5 Metagenomic sequencing

Metagenomic sequencing was performed to investigate the composition of ARGs in the samples. Before library construction, DNA was purified and concentrated on Microcon columns (MilliporeSigma, Burlington, MA, USA). DNA quantification was performed on a Qubit 3.0 fluorometer (Thermo Scientific, Waltham, MA, USA). Library preparation was completed through NEBNext® UltraTM DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) according to the Kit manual, and index codes were added to label reads of each sample. Briefly, sonication was used to fragment the DNA to a size of 350 bp. The fragmented DNA was then end-polished, A-tailed, and ligated with the full-length adaptor for further PCR amplification. The PCR products were purified using AMPure XP system (Beckman Coulter, Brea, CA, USA). The size distribution and quantity of each library were confirmed by Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and real-time PCR, respectively. The clustering of the index-coded libraries was performed on cBot Cluster Generation System (Illumina, San Diego, CA, USA) following its manual. After cluster generation, the libraries were paired-end sequenced (2×150 bp) on NovaSeq 6000 platform (Illumina, San Diego, CA, USA) according to the standard protocols of the platform. The DNA concentration and clean data amount are shown in Table S2.

2.6 Bioinformatics analysis

The demultiplexed raw amplicon reads from the Miseq runs were denoised and dereplicated into amplicon sequence variants (ASVs) using DADA2 v.1.10 (for prokaryote: trimming the first 30 nt, truncating the forward reads at 220 nt, truncating the reverse reads at 250 nt; for eukaryote: trimming the first 30 nt, truncating the reads at 240 nt) [30]. Taxonomic classification of the representative sequence for each ASV was done using the plugin q2-feature-classifier in QIIME 2 v.2020.2 against the SILVA rRNA gene database (132 release) in default parameters [31]. For metagenomic analysis, ARGs-OAP v.2.1 was employed to profile ARGs against Structured ARG reference database (SARG, v.2.0) in default parameters [32].

2.7 Data analyses

Cleveland dot plot and heatmap were generated by R 3.6.2 (Revolution Analytics, USA) with ggplot2 and pheatmap package, respectively. Origin Pro (2017, OriginLab, USA) was used for bar chart generation and principal component analysis (PCA). PCA was used to analyze the similarities and differences of microbial communities among different samples. Spearman correlation analysis was performed by SPSS 19.0 Statistics (IBM, USA) to examine the relationships between microbial communities and ARG subtypes, and the P-value < 0.05 were considered to be statistically significant. The ARGs abundance was normalized to that of 16S rDNA as 'ARG copies per 16S rDNA copy'.

To assess the environmental impact and health risks of airborne ARGs, ARGs absolute abundance (copies m^{-3}) was estimated by multiplying the ARGs abundance (copies/16S rDNA) by 16S rDNA concentration (copies m^{-3}), where the 16S rDNA concentration was measured by qPCR. Daily intake of ARGs (copies d^{-1}) was calculated by multiplying the inhalation rate (m^3 d^{-1}) by ARGs absolute abundance (copies m^{-3}). The breathing rate refers to the USEPA [33], and the air inhaled by the worker within 8 h of work is 12 m^3.

3 Results and discussion

3.1 Microbial community structure

3.1.1 Prokaryotes

The bacterial community composition is shown in Fig. 1. Proteobacteria dominated in all the six samples, with relative abundance ranging from 87.1% (JAO) to 97.8% (JAR). Actinobacteria was the second most abundant bacteria with relative abundance ranging from 0.7% (JAR) to 12.6% (JAO). In addition, Firmicutes and Bacteroidetes were also detected. Zothanpuia et al. [34] found that Proteobacteria, Actinobacteria, and Firmicutes were the main phyla in the leachate of municipal waste dumping site in Aizawl city, and served as significant repositories of ARGs. This phenomenon was also similar to the release pattern of bioaerosol from waste composting. The previous study of our research group found that Proteobacteria accounted for the highest proportion both in the composting aerosols of sewage sludge [35] and of vegetable waste [36], accounting for 82.0–98.5% and 24.7–82.2%, respectively.

For bacterial genera, the relative abundance of *Pseudomonas* was the highest, ranging from 48.8% (HPR) to 92.1% (JAR). *Pseudomonas*, as a pathogen widely present in landfilled waste, has been shown to be an important host of ARGs [37]. *Acinetobacter* was detected with the highest relative abundance of 37.4% in the waste reception hall of HP transfer station, while the relative abundances in the waste reception halls of HK transfer station and JA transfer station were 7.0 and 2.7%, respectively. Other relatively abundant bacteria include *Rhodococcus*, *Stenotrophomonas*, *Brevundimonas*, *Lactobacillus* and *Gordonia*.

Unlike previous studies [36] on the composting of vegetable waste, no archaea were detected in this study.

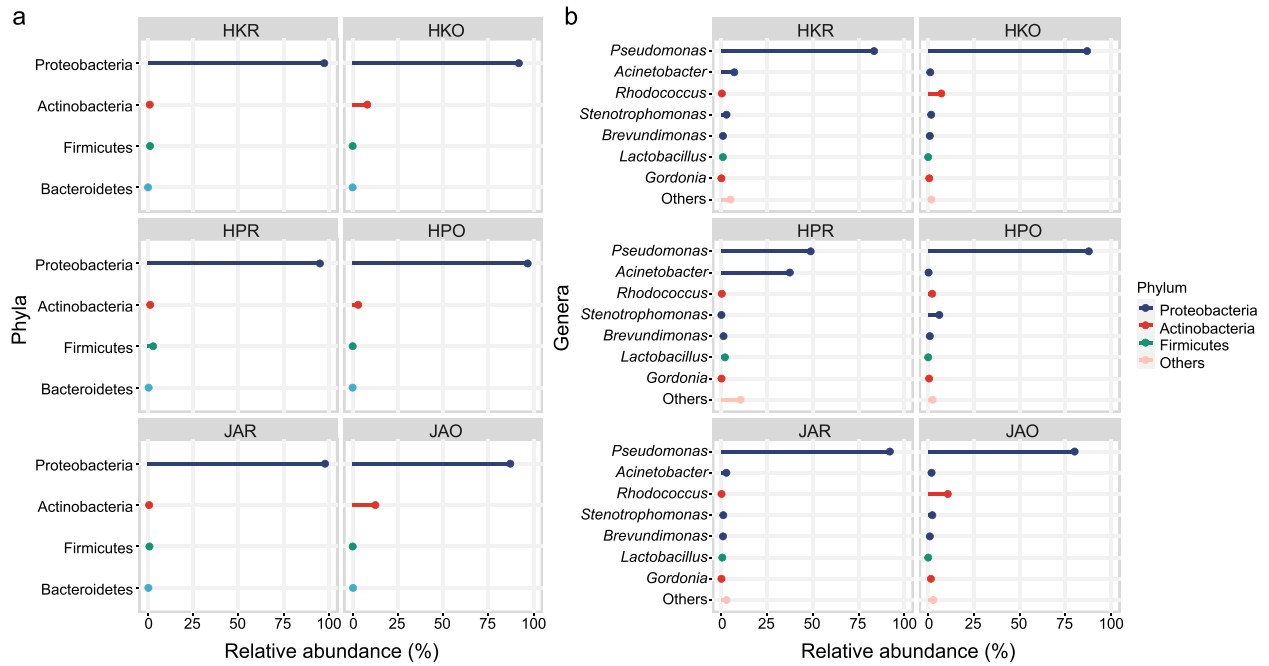

Fig. 1 Relative abundance of bacteria at phylum **(a)** and genus **(b)** levels at six sampling sites (waste reception halls of three transfer stations: HKR, HPR, JAR; top vents of three transfer stations: HKO, KPO, JAO). Genera belonging to the same phylum are represented by the same color as shown in the legend

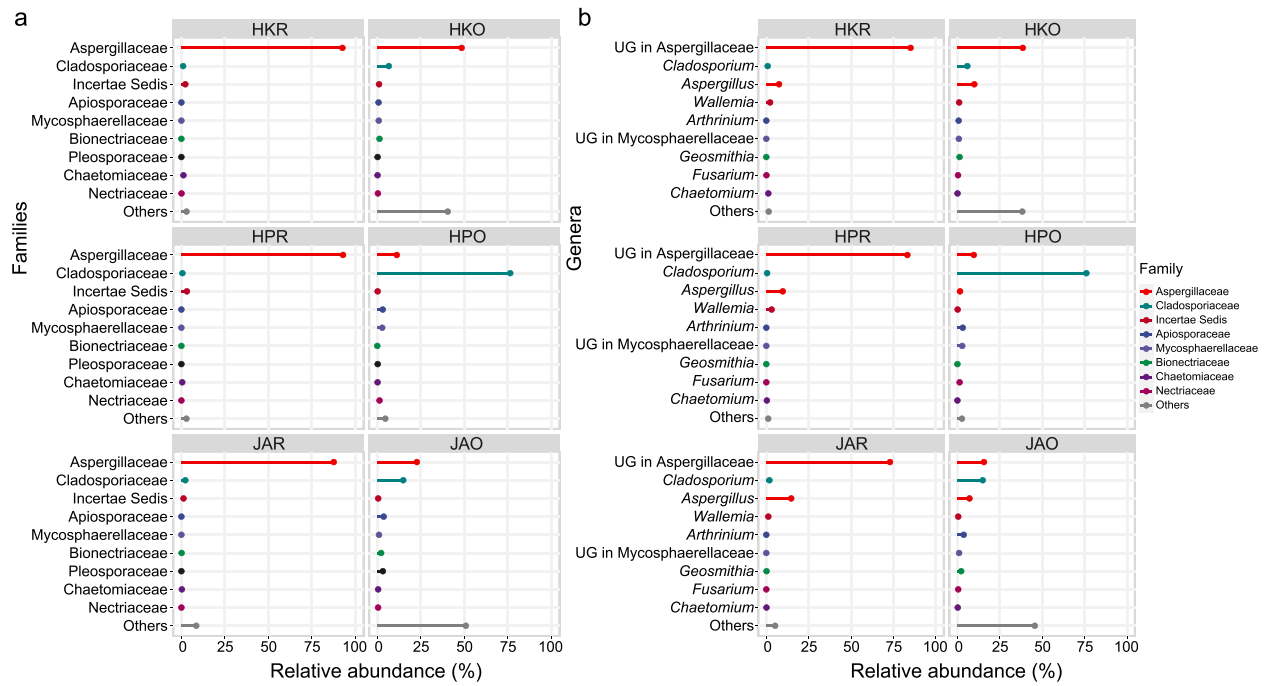

Fig. 2 Relative abundance of fungi at family **(a)** and genus **(b)** levels at six sampling sites (waste reception halls of three transfer stations: HKR, HPR, JAR; top vents of three transfer stations: HKO, KPO, JAO). Genera belonging to the same family are represented by the same color as shown in the legend. UG: Unclassified genera

3.1.2 Fungi

The composition of fungal communities is shown in Fig. 2. In the waste reception halls of the three transfer stations, Aspergillaceae dominated the fungal composition with relative abundance of 92.5% in HKR, 92.9% in HPR, and 87.5% in JAR. For the top vent, the relative abundance of Cladosporiaceae and Aspergillaceae was 76.4 and 11.2% respectively in HP. In the samples of HK and JA, Aspergillaceae was the dominant family, accounting for 48.4 and 22.7%, respectively. A variety of *Aspergillus* strains in Aspergillaceae have strong pathogenicity, among which *Aspergillus fumigatus* is the most important pathogen and has been proved to be the main cause of pulmonary diseases, such as aspergillosis and pulmonary tuberculosis [38]. *Aspergillus* and *Aspergillus fumigatus* have also been used as important quantitative indicators for health risk assessment [39].

At the genus level, the relative abundance of *Aspergillus* ranged from 1.5% (HPO) to 14.7% (JAR). *Cladosporium* was the most variable genus, with relative abundance ranging from 0.5% (HPR) to 76.0% (HPO). *Aspergillus* and *Cladosporium* are important microbial pollutants in the ambient air around landfills [40], and *Aspergillus fumigatus* has also been detected in large quantities in the air environment of open dumpsite [41]. *Arthrinium* was only detected in samples from the three top vents, in a range of 0.7% (HKO) to 3.6% (JAO). In addition, *Fusarium* and *Chaetomium* were also detected in the samples. The previous study found that *Galactomyces*, *Sclerotinia* and *Aspergillus* were the main aerosol fungi in the compost process of vegetable waste [36]. In the composting process of sewage sludge [35], *Cryptococcus*, *Fusarium* and *Cladosporium* were dominant. Compared with the above studies, *Aspergillus*, *Fusarium* and *Cladosporium* were common fungal genera, while *Arthrinium* and *Chaetomium* were unique fungal genera in the present study.

3.1.3 Variation among sampling sites

As shown in Fig. 3, PCA was used to further analyze differences in biological composition of each sampling site. Except HPR, the prokaryotic composition of the other five sampling sites was similar, especially in the waste reception halls of JA and HK. The composition of fungi in the three waste reception halls was similar, but the differences between the three top vents were huge, which was different from that of prokaryotes. There are two possible reasons for this phenomenon. First, the aerodynamics of different microorganisms are different because of their size, shape, and other properties. For example, fungi are usually larger or longer than bacteria. Second, the different odor treatment system of the transfer station could have different effects on diverse microorganisms. Overall, the composition of prokaryotes and fungi was relatively similar in the waste reception halls of the three transfer stations. This may be because the three transfer stations respectively located in three adjacent districts of Shanghai, with small differences in environmental factors, and the types of waste handled were the same.

3.2 Composition and abundance of ARGs

Metagenomic analysis showed that the main airborne ARGs types in the waste reception halls and top vents

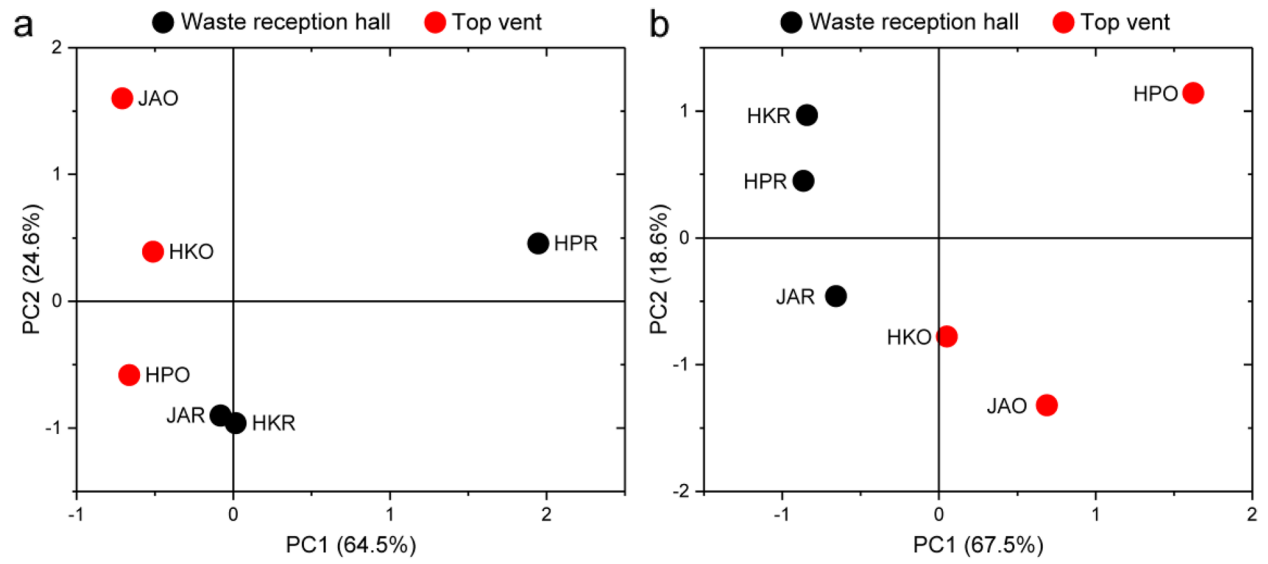

Fig. 3 PCA of prokaryotic **(a)** and fungal **(b)** community composition at the genus level

of the transfer stations were multidrug resistance genes (2.09 copies/16S rDNA, average hereafter), bacitracin resistance genes (0.59 copies/16S rDNA), sulfonamide resistance genes (0.14 copies/16S rDNA), macrolide-lincosamide-streptogramin resistance genes (0.09 copies/16S rDNA), quinolone resistance genes (0.09 copies/16S rDNA) and beta-lactam resistance genes (0.05 copies/16S rDNA) (Fig. 4a). Among them, multidrug resistance genes had the highest abundance at all sampling sites, ranging from 0.60 (HPR) to 4.37 (HKO) copies/16S rDNA. It was significantly different from the reported studies on transfer stations. In the study by Li et al. [13] using qPCR, multidrug resistance genes were not considered, and beta-lactam resistance genes were found to have the highest abundance in the air environment of the waste processing area. This indicates that the traditional PCR method may seriously underestimate the contamination degree of sampling sites. In studies of airborne ARGs through metagenomics, abundance of multidrug resistance genes was the highest in both hospital (0.10–0.15 copies/16S rDNA) [19] and wastewater treatment plant (0.33 copies/16S rDNA) [20] but still much lower than the results in this study.

Microorganisms harboring multidrug resistance genes show resistance to multiple antibiotics simultaneously. Multidrug resistance genes were also widely present in landfill leachate [42, 43]. The results of this study indicated that the presence of many different types of antibiotics in such a complex environment containing a variety of wastes provided environmental selection pressure for the generation and enrichment of multidrug resistance genes.

A total of 265 ARG subtypes were found in the sampling sites, and the subtypes with an average abundance of more than 0.01 copies/16S rDNA were selected for further analysis (Fig. 4b). The average abundance of bacitracin resistance genes *bac*A was the highest, ranging from 0.04 (HKR) to 1.36 (HKO) copies/16S rDNA. The second was multidrug resistance genes multidrug transporter, which ranged from 0.07 (HKR) to 0.88

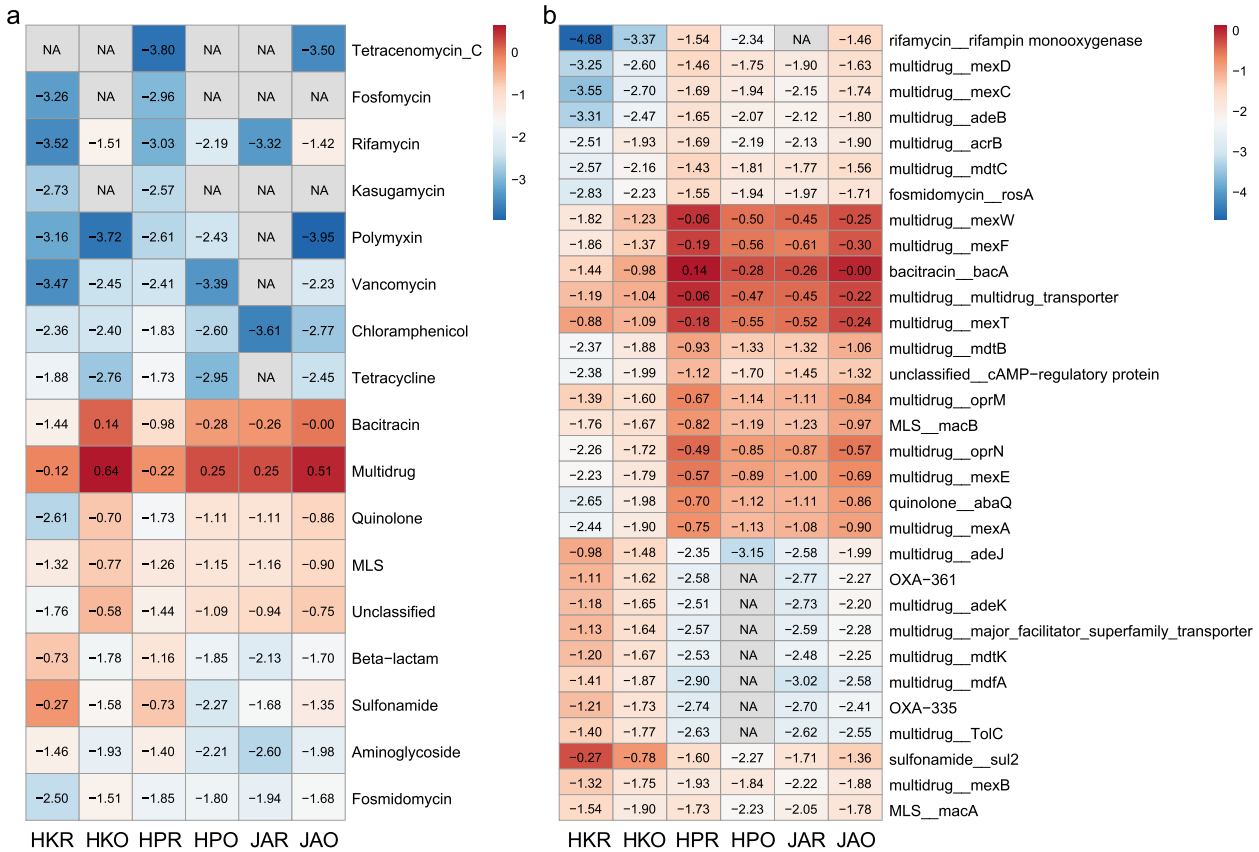

Fig. 4 Abundance [$\log_{10}$ (copies/16S rDNA)] of ARG types (**a**) and ARG subtypes (**b**) in the six sampling sites (waste reception halls of three transfer stations: HKR, HPR, JAR; top vents of three transfer stations: HKO, KPO, JAO). ARG Subtypes listed only 31 species with a sum of abundances greater than 0.05 copies/16S rDNA. MLS: Macrolide-Lincosamide-Streptogramin. NA: not detected

(HKO) copies/16S rDNA. Meanwhile, among the 10 ARG subtypes with the highest abundance, 7 were multidrug resistance genes. In the waste reception halls of HK and HP transfer stations, the abundance of sulfonamide resistance genes *sul*2 was the highest.

3.3 Correlation between ARGs content and microbial composition

The distribution and abundance of ARGs could be closely related to the bacterial community in the environment [44]. Therefore, 7 major bacterial genera and 40 airborne ARG subtypes were selected for spearman correlation analysis with the results shown in Fig. 5. We hypothesized if there was a strong ($\rho > 0.8$) and significant ($P < 0.05$) positive correlation between bacterial taxa and ARG subtypes, the correlation can be used to indicate the ARG hosts in the bioaerosols [45]. Five bacterial genera (*Gordonia*, *Rhodococcus*, *Lactobacillus*, *Acinetobacter* and *Pseudomonas*) were significantly correlated with 22 of 40 ARG subtypes ($P < 0.05$), indicating that these bacteria might contribute significantly to shaping the profile of ARGs in the bioaerosol. Specifically, *Gordonia* and *Acinetobacter* were significantly and positively correlated with 12 and 6 ARG subtypes, respectively, indicating that they may be the hosts of these ARG subtypes in the air. While *Lactobacillus* showed negative correlation with up to 13 ARG subtypes, *Acinetobacter* was also negatively correlated with two ARG subtypes, indicating that the emergence of these genera would have a negative impact on the formation and spread of ARGs. *Pseudomonas* with the highest relative abundance in this study showed a significant correlation with only one ARG subtype (*sul*1). No effect of *Stenotrophomonas* and *Brevundimonas* on the distribution of major ARG subtypes was found.

Fungal community, another important component of bioaerosols, also had an important influence on the distribution of ARGs (Fig. 6) [5]. Notably, Bionectriaceae was positively correlated with 12 ARG subtypes, with 6 species (*bac*A, multidrug_transporter, *mex*W, *mex*T, *opr*M, and *aba*Q) in the top 10 in relative abundance. The enrichment of 10 ARG subtypes such as *sul*2 then might be closely related to the high abundance of Chaetomiaceae. Overall, these results suggested that the distribution of ARGs was driven by the microbial community. Therefore, when the air in the waste reception hall of the transfer station was discharged through the odor treatment system to the top vent, the change in bacterial composition would also lead to changes in the distribution and abundance of ARGs.

3.4 Release characteristics of airborne ARGs from transfer station

Based on the above metagenomic results, the differences in the abundance of ARGs at the waste reception halls and the top vents of three transfer stations were compared. At all three transfer stations, the abundance of ARGs increased as they were released from the waste reception hall to the top vent (Fig. 7a). The index of HK transfer station increased from 1.64 to 6.50 copies/16S rDNA, an increase of nearly three times.

Figure 7b shows the content changes of the ten major ARG subtypes. The total proportion of these ten ARG subtypes in each sample was more than 50%. Except for *sul*2, other ARG subtypes had higher abundance at the top vent. Among them, quinolone resistance genes *aba*Q showed the greatest difference, and the abundance ratio reached 89.3 times at HK transfer station. Sulfonamide resistance genes *sul*2 had a higher concentration in the waste reception halls of HK and HP transfer stations. As the analysis in the section 3.3, *sul*2 did not show strong correlation with the main microbial taxa in the samples, which was completely different from the other nine ARG subtypes, which also meant that their vectors might be quite different. Under this assumption, when different vectors were treated by the odor treatment system, they showed different variation patterns, and the ARGs showed different release characteristics. Another possibility is that too many microorganisms commonly possessed this ARG.

Understanding the release characteristics of airborne ARGs is a prerequisite for taking measures to reduce environmental impacts and health risks. Unfortunately, there is no relevant study for reference and comparison at present. Studies have shown that extracellular DNA is a ubiquitous component that plays an important biological role in the microbial community [46], and ARGs have been detected in extracellular DNA [47]. The study on the release characteristics of airborne ARGs should not consider the only influence of microbial carriers. Further research is therefore needed. In general, although the release characteristics of different ARG subtypes were different, the overall abundance in the top vent was higher than that in the waste reception hall, indicating that the current odor treatment system played a certain role in leading to higher concentration of ARGs. Given the current lack of research on the removal of airborne ARGs, we call on more researches to pay attention to this issue.

3.5 Risk assessment

The concentrations of 16S rDNA and 18S rDNA at each sampling site were shown in Fig. 8a. For 16S rDNA, the contents of the six samples ranged from $5.49 \pm 0.82 \times 10^8$

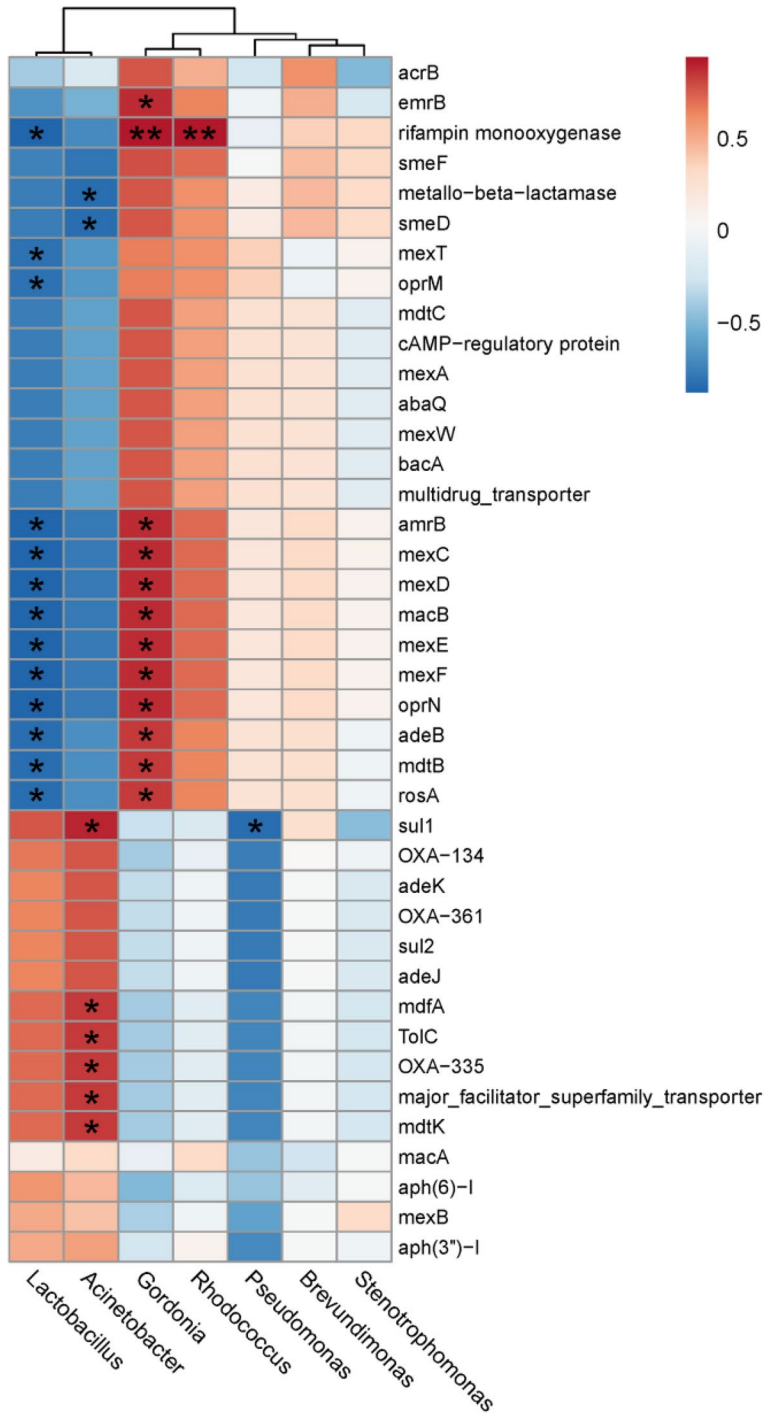

Fig. 5 Spearman correlations between ARG subtypes and the top 7 bacteria at the genus level. Red and blue indicate positive and negative correlations, respectively. Asterisks mean strong significant correlation (** $P < 0.01$) and significant correlation (* $P < 0.05$). The absolute values of the correlation coefficients are all greater than 0.8 in the places marked with asterisks

to $1.46 \pm 0.28 \times 10^9$ copies m^{-3}. For reference, the 16S rDNA level was about 10^5 copies m^{-3} for the environment around small MSW transfer stations [13] and 10^5–10^8 copies m^{-3} for composting plants [15, 48, 49]. In urban areas, the value is about 10^3–10^4 copies m^{-3} [50]. For 18S rDNA, the contents of the six samples

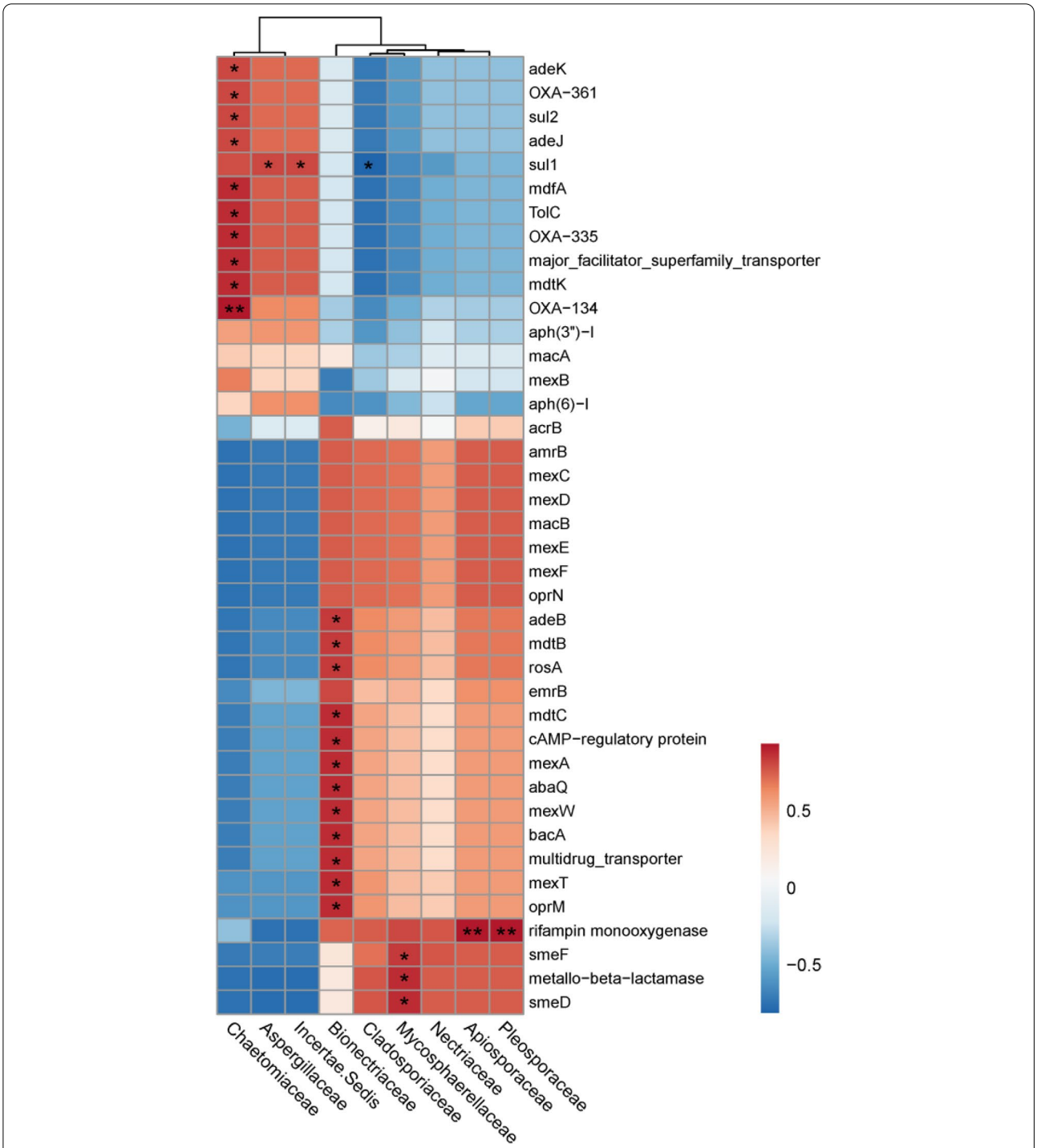

Fig. 6 Spearman correlations between ARG subtypes and the top 9 fungi at the family level. Red and blue indicate positive and negative correlations, respectively. Asterisks mean strong significant correlation (** $P < 0.01$) and significant correlation (* $P < 0.05$). The absolute values of the correlation coefficients are all greater than 0.8 in the places marked with asterisks

ranged from $2.23 \pm 0.38 \times 10^6$ to $7.28 \pm 0.22 \times 10^6$ copies m^{-3}. The concentration of 16S rDNA at the top vent of each transfer station was higher than that in the waste reception hall, and the concentration ratio of the two sampling sites at HK transfer station reached 2.5. The concentration of 18S rDNA was also higher at the top

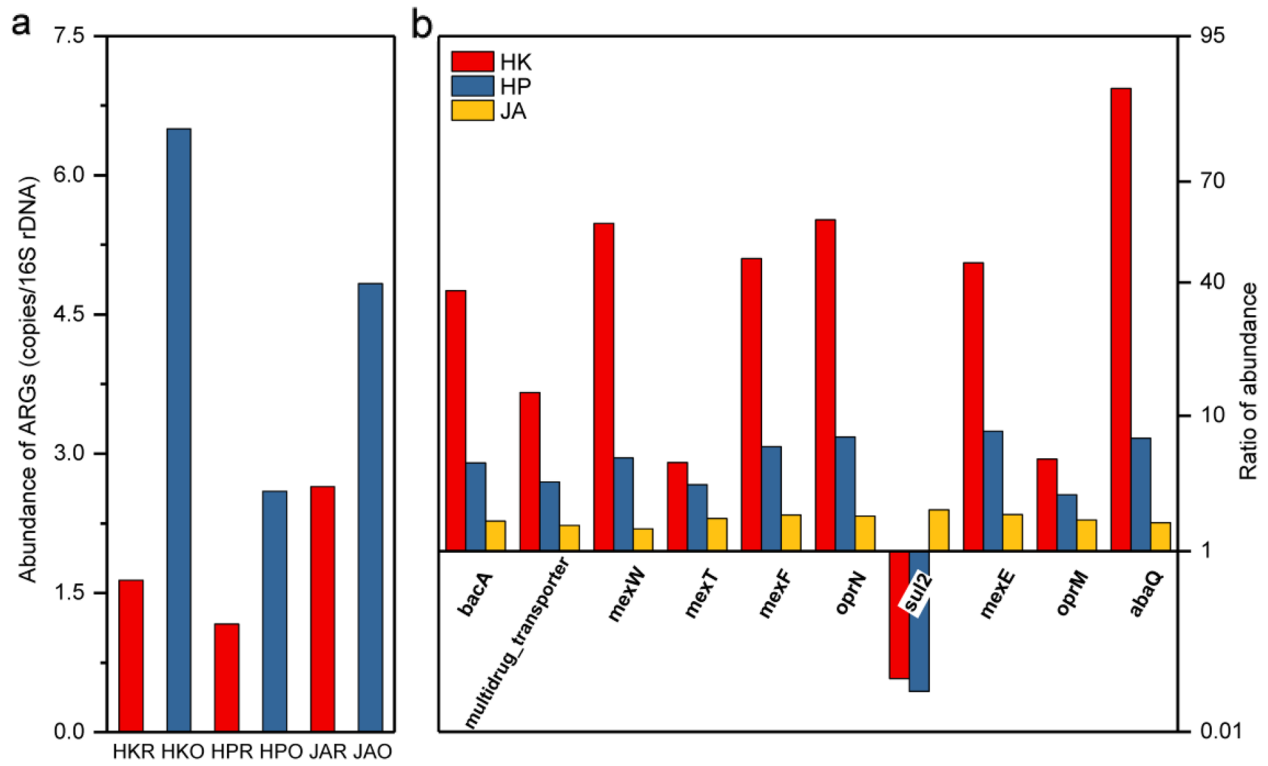

Fig. 7 Release characteristics of airborne ARGs. **(a)** Abundance (copies/16S rDNA) of ARGs at six sampling sites (waste reception halls of three transfer stations: HKR, HPR, JAR; top vents of three transfer stations: HKO, KPO, JAO). **(b)** The abundance ratio of the top vent to waste reception hall for the ten ARG subtypes with the highest average abundance

vent at HK and JA transfer stations, and the opposite was true at HP station. The direct connection of this was a sharp drop in the relative abundance of Aspergillaceae from the waste reception hall (92.9%) to the top vent (11.2%) at the HP transfer station.

To assess the possible health risks and environmental impacts of airborne ARGs, the daily intake of workers in the waste reception hall and the release from transfer stations into the surrounding environment were calculated (Fig. 8b). Each index contained the total amount

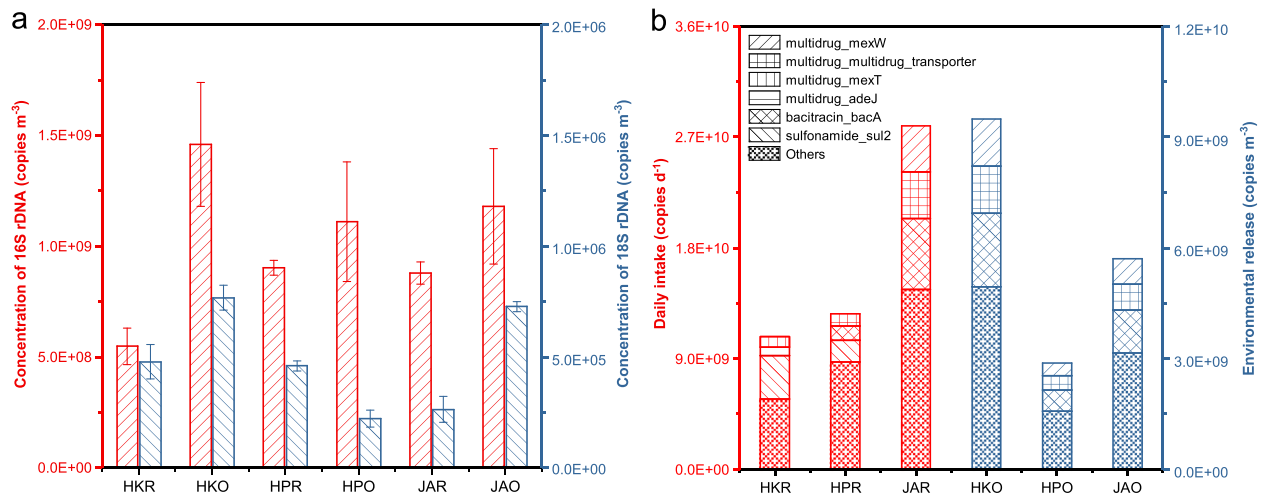

Fig. 8 **(a)** Concentration (copies m^{-3}) of 16S rDNA and 18S rDNA at six sampling sites (waste reception halls of three transfer stations: HKR, HPR, JAR; top vents of three transfer stations: HKO, KPO, JAO). Red and blue represent 16S rDNA and 18S rDNA, respectively. **(b)** Daily intake (red) and environmental release (blue) of ARGs, including total ARGs and the three subtypes most affected at each sampling site

and the three ARG subtypes with the greatest influence at each sampling site. The daily intake of total airborne ARGs of a worker ranged from 1.08×10^{10} (HKR) copies d^{-1} to 2.79×10^{10} (JAR) copies d^{-1}. However, in previous study of MSW treatment and disposal facilities, researchers found that the daily intake burden level of ARGs via particulate matter inhalation was comparable to that via ingestion of drinking water (10^6–10^7 copies d^{-1}) [13]. At the JA transfer station, the most intaked ARG subtype by workers was *bac*A, reaching 5.76×10^9 copies d^{-1}, which was 2–3 orders of magnitude higher than that in other studies [5, 13]. At HK and HP transfer station, the highest daily intake ARG subtype was *sul*2, with daily intakes of 3.52×10^9 and 1.78×10^9 copies d^{-1}, respectively. From the perspective of environmental release, HK transfer station is the highest, reaching 9.49×10^9 copies m^{-3}. The environmental releases of the other two transfer stations are 2.88×10^9 (HPR) copies m^{-3} and 5.70×10^9 (JAR) copies m^{-3}. The ARG subtypes with the highest release of the three transfer stations to the external environment were all *bac*A.

To sum up, although the odor treatment system of the transfer station meets the relevant standards for the treatment and discharge of odor pollutants, it does not have the desired effect on the removal of pathogenic microorganisms and airborne ARGs. Both the waste reception halls and the top vents contain high levels of disease-causing microorganisms and airborne ARGs, posing a health threat to the workers and the surrounding residents. Therefore, further measures are urgently needed to reduce the environmental release of pathogenic microorganisms and airborne ARGs from MSW transfer stations.

4 Conclusions

A total of 265 subtypes of airborne ARGs were detected in the six samples. The main subtypes were *bac*A, multidrug_transporter, *mex*W, *sul*2 and *mac*B. Multidrug resistance gene was the most abundant ARG type, which reflected the environmental selective pressure of microorganisms due to the coexistence of multiple antibiotics in the waste components of transfer stations. Correlation analysis showed that the distribution of ARGs was driven by the microbial community, but the main carrier of ARGs might not be the dominant population in aerosol microorganisms.

The concentration of 16S rDNA and the abundance of ARGs at the top vent of each transfer station were higher than those in the waste reception hall, suggesting that the current odor treatment system routinely used in transfer stations not only failed to remove potential pathogenic microorganisms and airborne ARGs, but also unexpectedly enriched the two kinds of pollutants. This has produced a great health threat to the workers and the surrounding residents. There is an urgent need for emission standards and more effective controls to reduce the resulting health risks.

Supplementary Information

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Additional file 1.

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Authors' contributions
Fan Lü: Methodology, Validation, Formal analysis, Writing - Review & Editing, Data Curation; Wei Wang: Formal analysis, Investigation, Writing - Original Draft, Visualization; Tianyu Hu: Investigation, Formal analysis, Visualization; Haowen Duan: Software, Visualization; Liming Shao: Supervision, Project administration; Hua Zhang: Resources, Validation; Pinjing He: Conceptualization, Methodology, Data curation, Writing - Review & Editing, Funding acquisition. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are available on request.

Declarations

Competing interests
The authors declare they have no competing interests.

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