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Pollution profiles of antibiotic resistance genes associated with airborne opportunistic pathogens from typical area, Pearl River Estuary and their exposure risk to human

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ABSTRACT

To reveal the selective pressures of near-shore human activities on marine and continental bioaerosols, the pollution profile and potential exposure risk of airborne pathogens and antibiotic-resistance genes (ARGs) in Pearl River Estuaries (113.52 °E, 22.69 °N), a transitional zone between marine and continental environments, were fully explored. The results showed that the total bacteria among bioaerosols varied largely with average pollution levels of 1.86×10^5 and 4.35×10^4 cfu m^{-3} in spring and summer, respectively, and were high than those of airborne fungi. The predominant aerodynamic diameters of bioaerosols were in respirable size range (< 4.7 μm), and the microbes communities' diversity and abundance varied significantly. Besides, many opportunistic pathogenic bacteria (*Burkholderia-Paraburkholderia, Staphylococcus* and *Acinetobacter*) and fungi (*Alternaria*, *Penicillium* and *Cladosporium*) were dominant in bioaerosol samples. Of 21 ARGs subtypes detected, the tetracycline resistance gene *tetA* was the most abundant, followed by aminoglycoside resistance gene and mobile genetic elements. Correlation analysis revealed that the changes of pathogens community contributed significantly to the prevalence of ARGs in bioaerosol. Based on the average daily dose rates of microorganisms and human direct intake of ARGs, health risk of bioaerosols from the Pearl River Estuaries were also evaluated. In summary, the presence of opportunistic pathogens and diversity of ARGs strengthens the call to consider the bioaerosol in air quality monitoring and risk assessment in the future.

1. Introduction

Bioaerosols comprised bacteria, fungal hyphae and spores, plant pollen, algae, as well as cell excretions or fragments (Fröhlich-Nowoisky et al., 2016) are important components of aerosol making up 30%–80% of the particulate matter (PM) in ambient air (Xie et al., 2018c). Among them, bacterial and fungal species are the most dominant components with the estimated amount emitted into the earth's atmosphere of 1.4–4.6 and 28–50 Tg $year^{-1}$, respectively (Heald and Spracklen, 2009; Woo et al., 2018). This means that high amount of pathogenic and/or non-pathogenic dead or alive microorganisms may exist in bioaerosol and would have major influence on human health and climate. For instance, bioaerosols are speculated to impact the hydrological cycle and climate by serving as nuclei for cloud droplets, ice crystals, as well as precipitation (Fröhlich-Nowoisky et al., 2016). Besides, biological aerosols are also caused major concern to human health by resulting in respiratory symptoms, infectious diseases, acute toxicity as well as cancers (Douwes et al., 2003).

The principal health risks were probably associated with respiratory symptoms as well as lung function impairment due to infection of pathogenic bacteria or fungi. For example, the inhalation of bacterial pathogens like *Escherichia coli, Klebsiella pneumonia*, and *Salmonella* spp. can lead to the destructive effects on human lungs (Gotkowska-Plachta et al., 2013). *Pseudochrobactrum* sp., *Chryseobacterium* sp., *Brevundimonas* sp. and *Yersinia* sp. from the aerosols of wastewater treatment plant were identified as human pathogenic bacteria (Wang et al., 2019). Besides, the inhalation of pathogenic viable airborne fungi *Candidia*, *Pneumocystis* spp. and *Cryptococcus* spp. were also found to be related to high mortality in immunocompromised individuals (Yamamoto et al., 2012). *Aspergillus fumigatus* were related to pulmonary infections and allergies of the workers in Wastewater Treatment Plants (WWTPs) (Viegas et al., 2014).

Given most airborne pathogens are multidrug-resistant (Dijkshoorn et al., 2007), the increased human respiratory diseases may also be

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associated with the increasing dissemination of antibiotic-resistant bacteria (ARB) as well as antibiotic-resistance genes (ARGs) through horizontal gene transfer of mobile genetic elements (MGEs) (Wang et al., 2014). By performing a global survey of ARGs, Lin et al. found that urban air is extensively contaminated by MGEs and ARGs, although different regions harbored varying health risks related to ARGs or ARB (Lin et al., 2018). The bacterial biota even possessed higher richness of ARG types (64.4) in Beijing smog as compared with certain occupational environments such as pharmaceutically polluted environments (38.9), wastewater/sludge (19.4) (Pal et al., 2016). Besides, Zhang et al. also pointed out that 45% of airborne bacteria isolated from the WWTP contained more than three antibiotics encoding genes and some pathogens were resistant up to 16 antibiotics (Zhang et al., 2018). This has undoubtedly increased intensive global concern and the risks to human health. Unfortunately, such information on the airborne ARGs pollution profiles and their inhalation health impacts is substantially lacked possibly attributed to the difficulty of capturing sufficient amounts of bioaerosol for ARGs identification and further quantification (Usachev et al., 2012).

Currently, the health risk assessment exposed to airborne bacteria and ARGs has not yet become a routine task due to no uniform standard method has been established (Liu and Bei, 2016). Only a few studies have been focused on the exposure risk assessment of bioaerosol on the bases of recommended models by the United States Environmental Protection Agency (USEPA) (Li et al., 2013). For example, the inhalation was found to be the main exposure pathway of workers in WWTPs to airborne bacteria with concentration range of 23–4878 cfu m^{-3} (Yang et al., 2019), and $PM_{2.5}$ exposure contributed to the total daily intake of ARGs on bioaerosol in the studied regions of China (Xie et al., 2019).

The bioaerosol pollution levels are significantly impacted by anthropogenic activities such as shipping transportation, aquaculture, and wastewater disposal. As a transitional zone between marine and continental environments, the bioaerosols in coastal estuaries not only reveal the contribution of land-based sources of ARGs and pathogens, but also the pollution profiles of marine sources microbes. For example, marine cyanobacteria are found to be a possible source of endotoxins in the bioaerosol of coastal areas (Lang-Yona et al., 2014). Therefore, herein, to reveal the effect of inland human activities and ocean on the airborne microorganisms, the bioaerosol emission levels, opportunistic pathogens diversity and abundance, and the pollution profiles of ARGs in Pearl River Estuaries located in a subtropical area were explored by Illumina sequencing and polymerase chain reaction (qPCR). Besides, inhalation exposure risks to ARGs as well as airborne microbes were also assessed by calculating human daily intake of ARGs and the average daily inhaled dose rates of microbes. The present work will provide information on public health risk assessment of bioaerosol exposure and human biodefense including epidemiological survey and risk modeling.

2. Experimental methods

2.1. Study sites and sample collection

The Pearl River Estuaries located on the southeast coast of China, have a subtropical maritime monsoon climate with annual average temperature of 21.4 °C-22.5 °C and precipitation of 1600–2300 mm (Huang et al., 2012). Sampling was conducted on 8 April 2019 and 8 August 2019 at eight sites across the Wanqingsha island (22°36′13″–22°44′1″ N, 113°30′35″-113°38′32″ E) located in the Nansha area, Guangzhou, China, respectively. As shows in Fig. S1, these sites were distributed in the industrial-influenced area (midstream of the river flooded 3 (R3M), downstream of the river flooded 6 (R6D) and 14 (R14D)), the residential-influenced sites (downstream of the river flooded 3 (R3D), midstream of the river flooded 6 (R6M) and 14(R14M)), and close to the inland and estuary area (midstream of the river flooded 1 (R1M) and 19 (R19M)), respectively. For tracking the source of bioaerosol, a sampling site was also set over the sea at 22.60° N-113.75° E.

Sampling of culturable bacteria and fungi was performed using a FA-1 six-stage cascade impactor (Applied Technical Institute of Liaoyang, China) at an air flow rate of 28.3 L min^{-1} for 5 min. The cut-points of each stage were: > 7 μm (stage 1), 4.7–7 μm (stage 2), 3.3–4.7 μm (stage 3), 2.1–3.3 μm (stage 4), 1.1–2.1 μm (stage 5) and 0.65–1.1 μm (stage 6). The total airborne microbes were also sampled with the sterilized polycarbonate membranes, which were placed on each plate of the impactor. The blank samples were collected using the same methods but without instrument operation. All air samples are collected at 1.5 m above the ground and approximately 500 m away from nearby major roads without surrounding obstacles. The detailed description of sampling methods was described in the SI.

2.2. The microbial community analysis by Illumina sequencing

The DNA in the membranes was extracted with the Rapid Soil DNA Isolation Kit (Sangon Biotech, China) according to previous study (Gao et al., 2018a) and used for Illumina sequencing with the primers 515F: 5′-GTGCCAGCMGCCGCGG-3′ and 806 R: 5′-GGACTACHVGGGTWTCTAAT-3′ targeting V4 hyper-variable region of the bacterial 16S rRNA gene, and ITS3F: 5′-GCATCGATGAAGAACGCAGC-3′ and ITS4R: 5′-TCCTCCGCTTATTGATATGC-3 targeting the internal transcribed spacer (ITS) region of the fungi, respectively. The detailed DNA extraction procedure and efforts made for improving the DNA quality, the methods used for PCR amplification, Illumina sequencing, and data processing were all described in SI. The community composition and relative abundance analysis of opportunistic pathogens were performed using the Bray-Curtis distance algorithm and visualized by Heatmaps. Besides, the principal coordinates analysis (PCoA) was used to reveal the differences between the microorganism communities' diversity and sampling location. High-throughput sequencing data of bacteria and fungi can be found in the NCBI Sequence Read Archive under accession numbers PRJNA633981 and PRJNA634430, respectively.

2.3. Detection of total microorganisms and airborne ARGs by qPCR

Specific primers in Table S1 were selected for amplifying the total bacteria and fungi (*legionella*). Amplification was carried out in triplicate on a BioRad CFX96 Touch system. The detailed PCR mixture and reaction conditions were shown in the SI. Using dilutions from a known content of genomic DNA, standard curve was established for each qPCR bacterial and fungal species. In addition, extracts from polycarbonate membranes were added into the subsets of diluted DNA for inhibition test, and no significant inhibition was observed (Hospodsky et al., 2010).

For evaluating ARG abundance in bioaerosol samples, 34 ARGs including genes resistant to β-lactam (*blaCTX-M*, *blaTEM* and *mecA*), aminoglycosides (*aadA*, *aadE aacA/aphD*, *str* and *sat*), tetracycline (*tetA*, *tetB*, *tetC*, *tetD*, *tetG*, *tetL*, *tetM*, *tetO*, *tetQ*, *tetS*, *tetT*, *tetX* and *tetW*), MLSB (*ereA*, *ermB*, *ermC*, *ermT*, *lnuA*, *lnuB*, *vatE* and *mefA*), sulfonamide (*sul1*, *sul2* and *sul3*) and multidrug (acrA and mexF), and 10 MGEs including integrons (*IntI1*, *IntI2* and *IntI3*), insertion sequences (*IS26*, *IS613* and *ISCR3*), plasmids (*traA* and *trbC*) and transposons (*merA* and *tnpA*) were all quantified in this work. These genes were measured due to the following four reasons: first, they can encode enzymes to resist the most frequently used antibiotics (sulfonamides, tetracyclines (Boxall et al., 2003; Chopra and Roberts, 2001), azithromycin (Arfaeinia et al., 2016b; Milaković et al., 2019) and ciprofloxacin (Arfaeinia et al., 2016a)) with broad-spectrum antibiotic effectiveness to prevent/treat human and animal diseases. Second, they are frequently detected in wastewater, sludge/soil, aquaculture and animal husbandry facilities (Zhao et al., 2018). Thirdly, MGEs contribute significantly to the transfer, retention and spread of antibiotics resistance (Li et al., 2016).

Final, they are the major shared airborne ARGs from typical bioaerosol emission sources including animal farms, wastewater treatment plant and the downtown area in the urban environment based on the metagenomic sequencing analysis (Yang et al., 2018). To cut down the interferences to the PCR reaction, all DNA samples were diluted to the contents ranging from 10 to 20 ng μL^{-1} according to the reference (Wu et al., 2017). qPCR protocols, quality control, as well as reaction systems were described in the SI, and primers were listed in Table S2.

2.4. The evaluation of risk

The exposure health risks to the bacteria and fungi on the bioaerosol were evaluated according to the USEPA recommended models. To be specific, the average daily dose rates (ADD, cfu (kg d)$^{-1}$) via inhalation were first assessed, then non-cancer exposure risks were assessed by dividing the ADD to the reference dose (RfD) of 500 cfu m^{-3} according to previous references (Yang et al., 2019; Yassin and Almouqatea, 2010). Detailed calculation equations were described briefly in Table S3 and SI. In the meantime, to assess the human exposure risk to ARGs via inhalation, the human daily intake of airborne ARGs (DI_{ARGs}, copy d^{-1}) could be estimated by multiplying the concentration of ARGs (copy m^{-3}) to inhalation rate (20 m^3 d^{-1}) according to the reference (Xie et al., 2019). The DI of opportunistic pathogens at the genus level can be estimated based on the following equation: $DI_{pathogens}$ (copy d^{-1}) = 16S rRNA gene concentration × relative abundance of pathogens × inhalation rate.

2.5. Statistical analysis

The meteorological parameters including wind direction, wind velocity, relative humidity and temperature) were collected from Guangzhou Meteorological Administration (http://www.tqyb.com.cn/). PM_{10}, $PM_{2.5}$, NO_2, SO_2, O_3 and CO are from Guangzhou Municipal Ecological Environmental Bureau (http://sthjj.gz.gov.cn/) and present in SI. Spearman correlation analysis was performed by SPSS 19.0 Statistics (IBM Corp, USA) to examine the relationships between bioaerosols and meteorological conditions, and p values < 0.05 were considered to be statistically significant. The relationships between environmental factors/ARGs and airborne pathogenic bacteria/fungi relative abundance and diversity indices were measured using canonical correspondence analysis.

To track the transport pathways of atmospheric particles that could influence the microbiological and chemical composition of bioaerosols in the study areas, backward trajectories were calculated using the Hybrid Single Particle Lagrangian Integrated Trajectory Model (HYSPLIT-4, http://ready.arl.noaa.gov/HYSPLIT.php) according to reference (Draxler and Rolph, 2013). The start point of the backward trajectory was set at the sampling site (22.60° N-113.64° E) with the altitudes of 100 m above the ground to estimate the accurate trajectories of atmospheric particles.

3. Results and discussion

3.1. The level and size distribution of airborne microorganisms

To understand the impact of bioaerosol in Pearl River Estuaries on global climate change, atmospheric microbial pollution and human health, the levels of total airborne microbes need to be revealed firstly. The results showed that the concentrations of airborne microorganisms varied significantly by season (ANOVA, $P < 0.001$). To be specific, in spring, the total concentrations of bacterial aerosols (1.03×10^5 –3.05×10^5 cfu m^{-3}) were significantly higher than those in summer (3.98×10^4 –1.83×10^5 cfu m^{-3}). Similarly, the levels of fungal aerosols in spring were also higher than those in summer (Fig. S2). A study conducted in Xi'an, which has a humid and rainy summer and short spring like Guangzhou, also found that the concentration of total airborne microbes in spring is higher than summer (Xie et al., 2018b). Other researchers revealed that the maximum concentration of airborne bacteria in the Qingdao coastal region occurred in spring and was 5 times higher than in summer. This might be mainly due to the environmental conditions including strong solar radiation, high ozone level and temperature in summer is hard for the survival and reproduction of these microbes (Li et al., 2011). Besides, overall levels of bacteria were higher than those of fungi with the maximum concentration detected in R3M and R14M in spring and summer, respectively. Besides, R3M also has the highest concentration of fungi, suggesting the severe bioaerosol pollution in the midstream area. It is not surprising attributed to stronger anthropologic activity in midstream area in the center of the island. Previous study was demonstrated that airborne microorganisms were originated from various activities including waste processing, wastewater treatment, agricultural and farming activities, industrial processes (Mirskaya and Agranovski, 2018). That is, the increased microbes' sources from human activities will result in higher concentrations of bioaerosol in the midstream area.

The bioaerosols aerodynamic diameters determine their potential to deposit into which regions of the lungs, such as trachea, bronchia, or alveoli; therefore the human health risks from bioaerosols are also related to their particle sizes (Brągoszewska et al., 2017). As shown in Fig. 1, the size distribution of bacterial aerosols demonstrated a bimodal pattern, with two peaks at 2.1–3.3 and 1.1–2.1 μm. Whereas the highest proportions of airborne fungi in the spring (29.8%) and in the summer (48.1%) ranged 2.1–3.3 μm, followed by 1.1–2.1 μm (21.5%) and 3.3–4.7 μm (19.5%), respectively. This difference can be attributed to the larger size of fungi than bacteria (Ghosh et al., 2015). It is also interesting to found that more than 70% of microbial aerosols were in the respirable size (< 4.7 μm) and thus may have high inhalation risk by depositing either into the upper or lower respiratory tract. The contributions of the coarse particles (> 2.1 μm) were reached 63.5% and 69.2% in spring, and 67.0% and 79.4% in summer for the airborne bacteria and fungi, respectively, suggesting that these microorganisms mainly existed in coarse particles. This result is in accordance with previous study that the airborne microbes were primarily in the coarse particle size (> 2.1 μm) with a proportion of 71.5% (Dong et al., 2016). There are two possible explanations: first, rough aerosol particles may indirectly boost airborne microbial abundance and diversity by conferring protection to them from severe atmospheric condition (e.g. UV irradiation); second, they may serve as the energy and carbon sources for airborne microorganisms (Clements et al., 2014). Thus, further exploring microbial communities of bioaerosol are helpful to figure out the reason for the different size distribution.

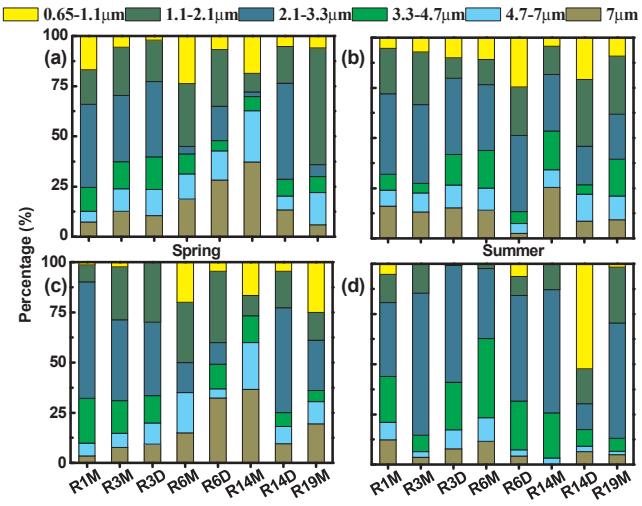

Fig. 1. The size distribution of bacteria and fungi in air samples collected from 8 different sampling sites in spring (a, c) and summer (b, d).

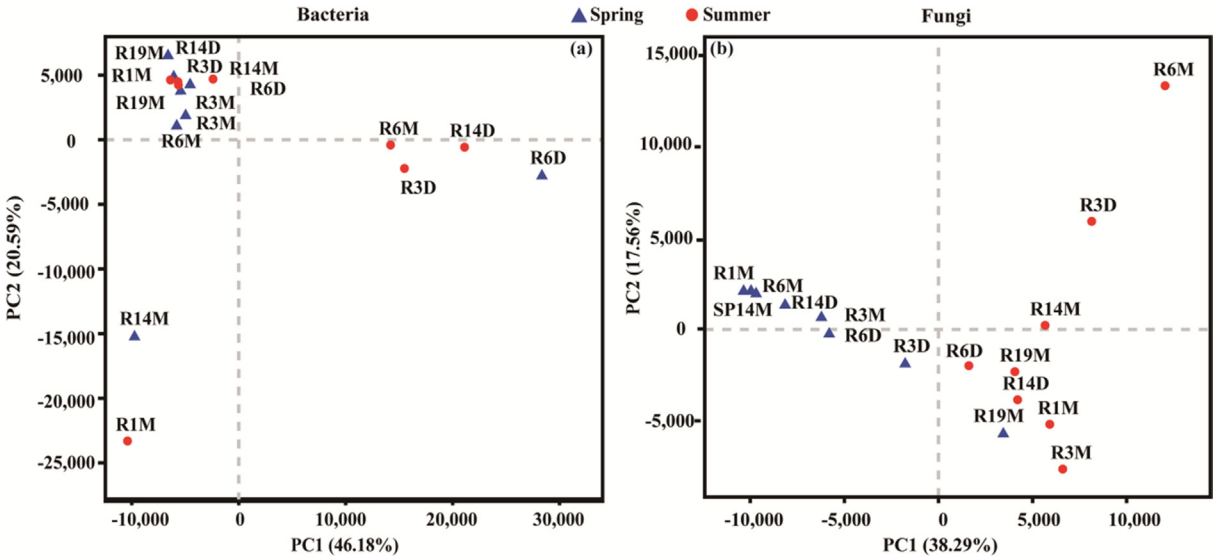

Fig. 2. Principle component analysis plot of airborne bacteria (a) and fungi (b) from 8 different sampling sites.

3.2. The abundance and diversity of airborne pathogens and ARGs

The community composition of the bioaerosols varied significantly throughout the study period. The Shannon index of the bacteria was higher than that of fungi, indicating the alpha diversity of the bacterial community was greater than that of fungi (Table S4). Moreover, the microbial richness and diversity were also higher in summer than that in spring based on the Chao1 and ACE indices. This might be varied with the situations of meteorology especially temperature, which is far higher in summer (34.4 °C on average) than that in spring (27.6 °C on average) (Table S5). Coincidentally, Bowers et al also reported that temperature was the possible meteorological factor significantly correlated with community composition of bioaerosol (Bowers et al., 2012). As shown in Fig. 2, the communities of all the samples were further classified into different clusters based on the PcoA analysis. The first two components PC1 and PC2, explained for 46.2% and 20.6% of the data variation for the bacteria, whereas for fungi the variations were 38.3% and 17.6%, respectively. Except for R6M, R14D, R3D and R6D, the other bioaerosol samples were cluster together, indicating they have very similar bacterial communities (Fig. 2a). Whereas for fungi, a clear separation was observed, revealing a significant shift of the overall fungi community between summer and spring.

At the phylum level, Firmicutes and Proteobacteria were found to be the two most dominant bacteria in Pearl River Estuaries, followed by Actinobacteria (Fig. S3a). While the Firmicutes made up more than 97% of all bacterial reads in marine bioaerosol (Table S6), suggesting that Firmicutes were possibly from the ocean. Previous study also demonstrated that Firmicutes were the most abundant bacteria found over the Mediterranean Sea (Mescioglu et al., 2019). Higher relative abundance of Proteobacteria and Actinobacteria might suggest higher health risk regarding Proteobacteria were important hosts of ARGs (Qian et al., 2016), while Actinobacteria are antibiotics producing bacteria, usually showing self-resistance and multi-resistance (Su et al., 2015). At genus level, *Burkholderia-Paraburkholderia* and *Bacillales* were found to be the two main dominant pathogens (Fig. 3a) and high abundance of *Staphylococcus* was detected in R14M (87.4%) and R1M (79.7%). Besides, R6M (38.4%) was rich in *Burkholderiales*, while 53.9% and 33.8% of *Listeria* and *Bacillus,* respectively, were found in R19M. *Pseudomonas*, *Mycobacterium*, *Salmonella*, *Shigella*, *Acinetobacter*, *Streptococcus*, *Clostridium* and *Corynebacterium* were also detected with relative low abundances but were demonstrated to be harmful regarding their ability to cause human diseases. For example, *Pseudomonas aeruginosa*, as an opportunistic human pathogen, can infect the wound in burns or trauma sites. Besides, *Acinetobacter* can also cause lung infection leading to pneumonia and Sepsis. *Corynebacterium diphtheriae* is responsible for diphtheria and cutaneous infection (Lu et al., 2018). Overall, the abundance of bacterial pathogens in midstream sites is higher than that of the downstream sites. This is coincided with an increased anthropogenic impact in the midstream area, indicating that more attention should be paid to these areas especially in spring.

Likewise, the airborne fungi communities were also significantly distinct from one another (Fig. S3b). Ascomycota was found to be the dominant phylum making up on average of 84.3% (spring) and 54.6% (summer) of the fungal sequences; while Basidiomycota occupied 5.8% (spring) and 11.1% (summer). The high abundance of Mucoromycota in summer at R3M, R3D and R6M may responsible for the different composition of fungi communities in spring and summer. At the genus level, *Cladosporiu* was found to be the most abundant genus in spring except for R19M (*Chaetomium*) and R3D (*Setophaeosphaeria*). While in summer, *Choanephora* (R3D, R6M and R14M) and *Blakeslea* (R3M) were the most abundant genera (Fig. 3b). Besides, the dominant genera showed allergenic and pathogenic effects on humans also included *Alternaria*, *Cladosporium*, *Curvularia*, *Arthrinium*, *Aspergillus* and *Penicillium*. To be specific, *Alternaria* in the hypersensitive patients' nasal sinuses can cause allergic fungal sinusitis. *Aspergillus fumigatus* can cause allergic bronchopulmonary aspergillosis. Besides, *Schizophyllum* belongs to Basidiomycota being reported able to cause maxillary sinus infection (Yamamoto et al., 2012). Overall, similar to bacteria, the total abundance of fungal pathogens was higher in spring and midstream area than in summer and downstream.

Except for pathogens, the airborne ARGs could also be directly emitted from ARGs-carrying bacteria or their re-aerosolization via different human activities or winds (Lin et al., 2018). qPCR results demonstrated that up to 21 ARG subtypes were detected and the absolute abundance of ARGs was much lower in spring (5.7×10^7 copies m^{-3}) than in summer (1.1×10^8 copies m^{-3}) (Fig. S4). To be specific, in spring, the midstream area has higher ARG abundance than that of the downstream area and the highest abundance was found in R14M, suggesting that midstream may cause higher health risks to the surrounding residents. While in summer, the ARGs concentration showed an opposite trend with higher diversity found in downstream area. This is probably because in spring the wind direction is onshore wind (southeast or south), while in summer the winds originated mainly from the north (northwest) (Table S5). Higher frequency of ARGs was detected near downwind versus upwind of polluted sites (e.g. poultry farms) (Sancheza et al., 2016). Besides, backward air trajectory analysis

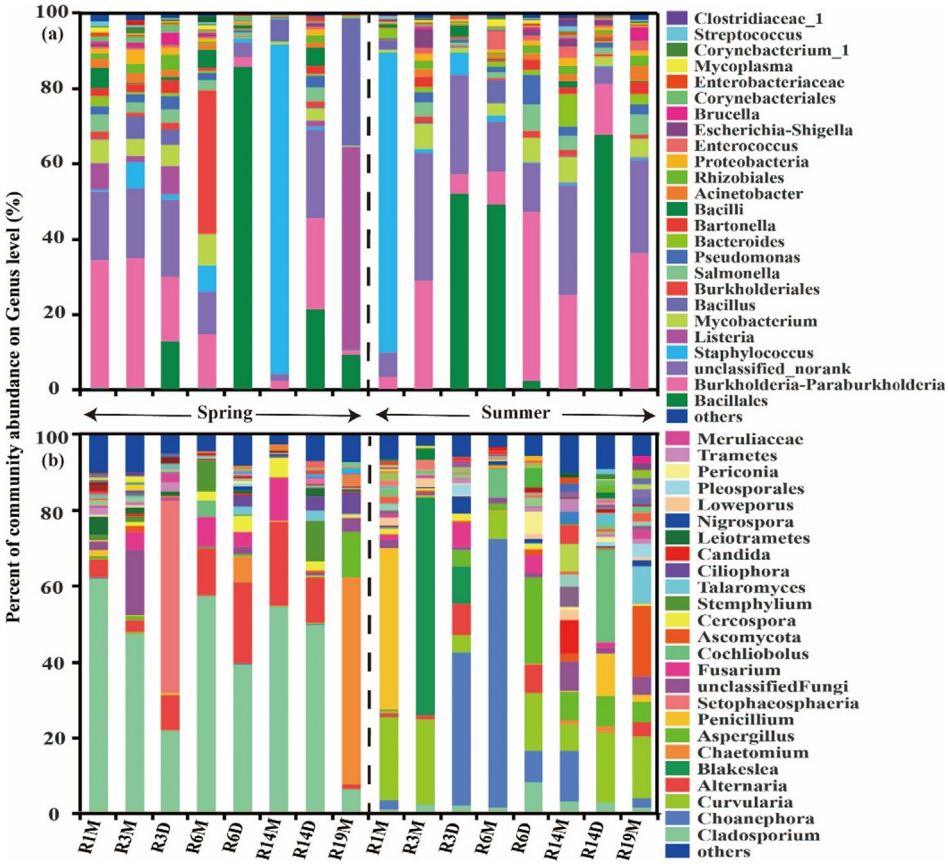

Fig. 3. The community compositions of airborne pathogenic bacteria (a) and fungi (b) at the genus level in spring and summer.

obviously showed various sources of bioaerosol during sampling period (Fig. S5). That is, in spring, with the influence of subtropical monsoon climate, the bioaerosols in Pearl River Estuaries mainly changed along the track of marine motion. Whereas, in summer, they were from the continental area under the influence of anthropogenic activity, and therefore resulted in higher ARGs abundance and diversity.

Among them, the most frequently detected ARGs were related to tetracycline resistance, comprising 50.4% and 59.4% of genes identified in spring and summer, respectively. Other frequently detected ARGs were aminoglycoside resistance genes and MGEs. Specifically, *tetA* and *tetB* were the most abundant ARGs related to tetracycline resistance, while *Sat* was a major aminoglycoside resistance gene in particular at R14M and R14D probably due to the influence of nearby seafood market or aquafarm (Fig. 4). Strains (*Escherichia coli*) isolated from commercial fish and seafood were resistance to *sat* and *aadA* genes (Ryu et al., 2012). This suggested that aquaculture process would produce a significant amount of aminoglycoside resistance bioaerosol (Chen et al., 2018). This result was also supported by previous studies demonstrating that ARGs related to tetracycline, aminoglycoside, and beta-lactam were the dominant ARG types in air samples (Gao et al., 2018a; Hu et al., 2018; Ouyang et al., 2020). For MGEs, high abundance of *IS613* and *ISCR3* were detected, especially in R1M, R3M and R14D, indicating the necessity and importance of monitoring MGEs in air. This is because transposases encoded by MGEs play important role in the evolution and proliferation of various ARB (Zhu et al., 2017). Overall, the relative ARG abundance in summer was higher than spring especially at the sites of R14 and R19, probably due to the selective pressure imposed by the nearby large aquatic products markets. Given ARGs could transmit among bacterial species as well as people, their high abundance in this study will increase the human susceptibility to the bacterial resistance, and therefore would inevitably result in high risk of "second-hand" inhalation of ARGs for the residents.

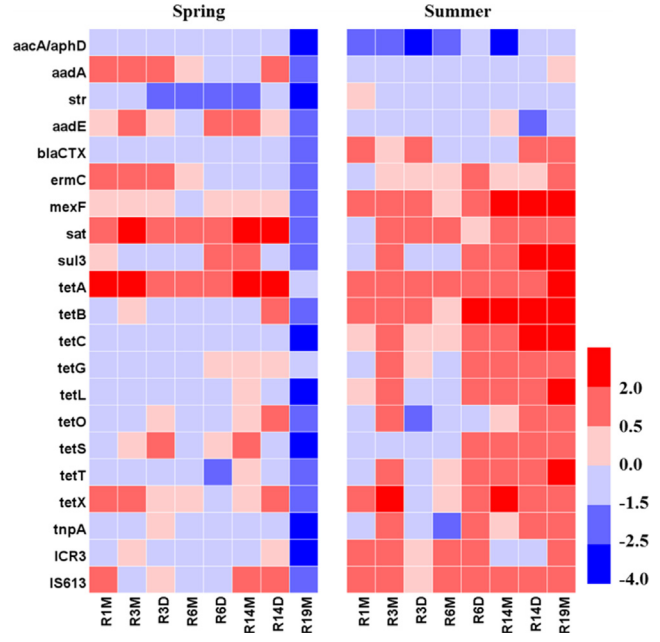

Fig. 4. Heatmap showing the relative abundance of different ARG types in air samples collected in spring and summer. Value plot is the logarithm transformed proportion of each ARG to 16S rRNA copy numbers.

3.3. Correlations of meteorological factors and ARGs with airborne pathogens

The situations of meteorology, including relative humidity (RH), temperature, ozone (O_3) level, and wind speed might influence the

seasonal variations of bioaerosol by impacting the survival of microorganisms (Styliani and Eleftheria, 2007). In this study, the airborne pathogens were positively correlated with RH ($p < 0.05$), while negatively associated with wind velocity, temperature and ARGs ($p < 0.05$). This is because the appropriate temperature (27.6 °C on average) and RH (77.8% on average) would lead to the accumulation of airborne bacteria (Frankel et al., 2012; Kozdrój et al., 2019), while dilution of ventilation would reduce bioaerosol concentrations. Specifically, in the point of bacteria, temperature and O_3 had a positive action on *Enterobacteriaceae*, *Acinetobacter* and *Escherichia-Shigella*; while RH had negative effect on these genera (Fig. S6a). This is in according with previous study that the concentrations of airborne total aerobic bacteria fluctuated with fluctuations of air temperatures and RH (Islam et al., 2019). For fungi, temperature showed a positive correlation with genera including *Nigropora*, *Aspergillus, Ascomycota* and *Curvularia*, while RH was positively related to *Alternaria*, *Cercospora*, *Cladosporium*, *Ciliophora*, *Leiotrametes* and *Stemphylium* (Fig. S6b). Previous study also demonstrated that meteorological parameters were positively correlated with airborne fungal spore such as Alternaria and Cladosporium (Grinn-Gofron et al., 2018, 2016). Overall, RH and wind speed have a greater effect on the distribution of fungi communities in spring, while temperature has significant effect on bioaerosol samples collected in summer, demonstrating the importance of meteorological conditions on the prevalence of airborne pathogenic microbial communities.

Besides, the distribution and abundance of ARGs were also closely related to the bacterial community especially pathogens in the environment (Forsberg et al., 2014). As the spearman correlation Heatmap in Fig. 5 illustrated, 20 of the 30 top genera were significantly correlated with ARGs ($p < 0.05$), demonstrating that these genera may contribute significantly to shape the profiles of ARG of bioaerosol. To be specific, *Escherichia-Shigella* was positively related to 10 ARGs. The high abundance of *tetB, mexF* and *tetC* possibly attributed to high distribution of *Escherichia-Shigella*, *Enterococcus*, *Clostridiaceae* and *Bacteroides*. Total 13 pathogens were positively related to *tnpA*, suggesting that they might acquire resistance via MGEs. Besides, 13 genera were found to be positively correlated with *tetX*, while 16 genera were related to *ermC*. This finding is accordance with a previous study that bacterial compositions can significantly affect the dynamic of ARGs (Su et al., 2015). We hypothesized if there was a strong ($r > 0.65$) and significant ($P < 0.01$) positive correlation between microbial taxa and ARGs, the correlation can be used to indicate the ARG hosts in the bioaerosols (Li et al., 2015). And the strong significant association between *Escherichia-Shigella*, *Burkholderia-Paraburkholderia*, *Enterococcus, Clostridiaceae* and *Bacteroides* with ARGs especially tetracycline resistance genes suggested they may be the possible host of these ARGs resulted in elevated abundance of ARGs in the airborne samples. However, negative correlation between *Bacillus*, *Listeria* and *Bacillales* and ARGs showed that the appearance of these genera will pose negative effect on ARGs formation and dissemination.

As another important component of bioaerosol, the fungi community also contributed significantly to the profiles of ARGs. As shown in Fig. S7, the high abundance of *Cladosporium* was positively related to *aadA, aadE* and *sat*, while negatively associated with *blaCTX, mexF* and *tetC*. The enrichment of *tetA* may be due to the high abundance of *Penicillium* and *Stemphylium*. *Ascomycota*, *Curvularia* and *Hyphodontia* were also found to be the possible hosts of multidrug (*mexF*) and tetracycline resistance genes (*tetB*, *tetC*, *tetT* and *tetX*) and MGEs. Overall, these results demonstrated that the distribution of ARGs was driven by microbial community. However, due to some environmental bacteria harbor intrinsic resistance, while others can acquire antibiotic resistance during the transmission of bioaerosol (Xie et al., 2019), in future research endeavors, more work should be done to elucidate the intrinsic and acquire resistance of the bioaerosol and their potential influences on human and environmental health.

3.4. Human exposure risk of airborne cultural microorganisms

Due to our above result has revealed that the majority of airborne

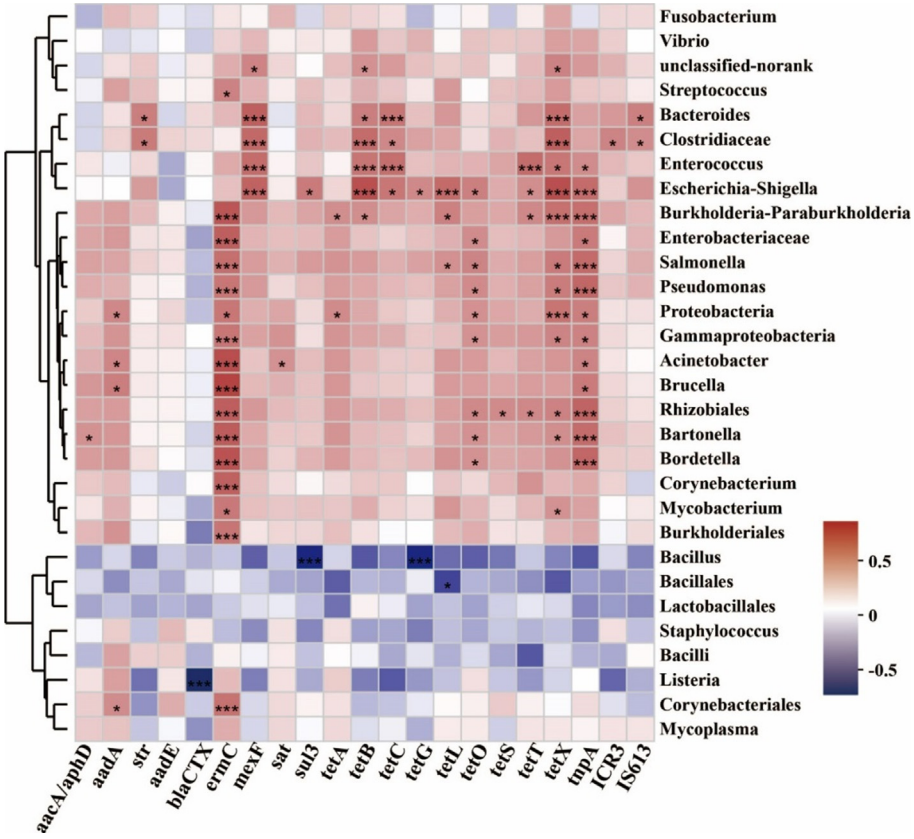

Fig. 5. Spearman correlations between ARGs and the top 30 airborne pathogenic bacteria at the genus level. One, two and three asterisks were used when $0.01 < p^* \leq 0.05$ (relatively strong correlation), $0.001 < p^{**} \leq 0.01$ (strong correlation), and $p^{***} < 0.001$ (significant strong correlation), respectively. The color transition from dark blue to dark red represents relative abundance of the community from low to high. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

microorganisms were in the range of respirable size (< 4.7 μm), it is of significance to evaluate the human exposure risk to airborne cultural microorganisms considering they have great potential to be inhaled into the lower airway of humans and resulted in the negative health effects like asthma or allergies (Gao et al., 2018b). According to the models recommended by USEPA, detection of atmospheric concentration of cultural bacteria and fungi was the prerequisite for the calculation of health risk. As shown in Fig. S8, the culturable bacterial level (303–2678 cfu m^{-3}) was higher than those of fungi (141–1554 cfu m^{-3}). The average concentration of bacteria (1186 cfu m^{-3}) and fungi (737 cfu m^{-3}) in spring were also higher than in summer. This is probably due to the following two reasons: firstly, the damage of strong UV irradiation as well as the cleansing of frequent rain during the summer (Ulevičius et al., 2004). Secondly, given sampling sites were surrounded by farmland, the increased microbe sources from crop cultivation and livestock farming in spring could also result in elevated bioaerosol concentration. Besides, significant variation ($p < 0.05$) was observed between the bioaerosols collected from midstream and downstream of the river flooded (Table S7). To be specific, in spring, R3D (2678 cfu m^{-3}), R6D (827) and R14D (1746 cfu m^{-3}) harbored higher concentration of culturable bacteria than R3M (1004 cfu m^{-3}), R6M (565 cfu m^{-3}) and R14M (304 cfu m^{-3}), respectively; while the highest fungi concentration was detected at R14D (1555 cfu m^{-3}), followed by R3D (1350 cfu m^{-3}) (Fig. S8a and c). Furthermore, in summer, R14D and R6D have higher airborne bacteria than other sites (Fig. S8b), suggesting that the bioaerosols in downstream were higher than that of the midstream area. One possible explanation is that the aerosol from the downstream areas will be more affected by the marine sources and long-distance transport of microbes than that of the midstream area. Besides, the downstream area is highly polluted by surrounded heavy industries such as brewery, textile dyeing industry and oil products industry. This is consistent with previous study that the relative abundance of microbial allergens and pathogens increased with increase of PM concentration (Cao et al., 2014).

Then, the health risks via inhalation of airborne microorganisms were measured. As Fig. 6 shows, the exposure risks for airborne bacteria and fungi changed with the seasonal variation in airborne microorganism concentration. In brief, the HQs were higher in the downstream area and in spring with high airborne microbes' concentration. For example, the highest health risk of airborne bacteria via inhalation was detected in R3D (0.34) in spring, follow by R14D (0.22). Similarly, the highest HQ of airborne fungi also occurred in spring, coinciding with the highest airborne concentrations. However, it has to mention that, the maximum HQs of airborne bacteria and fungi were lower than 1, suggesting that the inhalation risks in the Pearl River Estuaries can be neglected. Previous study also showed that the inhalation risk for the adults in the Qingdao were less than 1, no matter it is in heavily or lightly polluted days (Gong et al., 2020). This suggested the inhalation risks for humans in the ambient environment are possibly negligible. However, the HQs of airborne bacteria and fungi in the aerobic tank of WWTP with maximum concentration of airborne bacteria (1.00×10^4 cfu m^{-3}) and fungi (1.44×10^4 cfu m^{-3}) is higher than 1 (Han et al., 2019) indicating potential adverse health risks are of concern. Therefore, more attention should be paid to these highly polluted areas of bioaerosol especially during the outbreaks of deadly respiratory diseases.

3.5. Human exposure risk of airborne pathogens and ARGs

Long term exposure to airborne opportunistic pathogens might increase respiratory tract infection risk and subsequently lead to the cardiopulmonary diseases, which, in turn, might result in ineffective cure because of the antibiotic resistance. By evaluating the human inhaled risk of airborne pathogens, we found that the DI of opportunistic pathogens in spring was significantly higher than those in summer. The maximum DI level of *Listeria* was up to 9.1×10^8 copies d^{-1}, followed by *Bacillales* (1.6×10^8 copies d^{-1}) and *Bacillus* (5.7×10^8 copies d^{-1}) (Fig. S9). These DI levels are even much higher than *Bacillus cereus* (10^4-10^6 copies d^{-1}), an opportunistic pathogen carrying ARGs, found on the municipal solid waste treatment system (Li et al., 2020b). This suggested that bioaerosol from the estuary area might also have as higher health risk as landfill especially in view of their high abundance of opportunistic pathogens, such as, *Listeriosis,* which could cause fever and diarrhea as other foodborne germs.

Except for the pathogenic bacteria, the emergence and persistence of extracellular ARGs in the air and water are also very critical for antibiotic resistance spread, given ARGs can be horizontal transfer via conjugative plasmids or by uptake of extracellular DNA (Chen et al., 2019; Li et al., 2020a). Thus, it is also urgent to assess the exposure risk of the total daily intake of ARGs associated with bioaerosol to reveal the implications of airborne transmission of ARG to human health. As shown in Fig. 7, the health risk exposed to ARGs was found to be higher in summer than in spring. To be specific, the intake of *sat* (in spring) and *tetA* (in summer) through inhalation were found to be higher than those of other ARGs. Besides, the estimated daily intake of genes resistance to beta-lactam (*blaCTX)*, tetracycline (*tetB)* and MGEs (*ISCR3* and *IS613)* in summer were as high as 10^8 copies d^{-1}, and even the macrolide resistance genes (*ermC*) was up to 10^7 copies d^{-1}. These levels were all higher than the daily intake of ARGs by drinking water and ingestion of agricultural soil in Nanjing (Xie et al., 2018a), suggesting that inhalation was another important exposure pathway to ARGs for

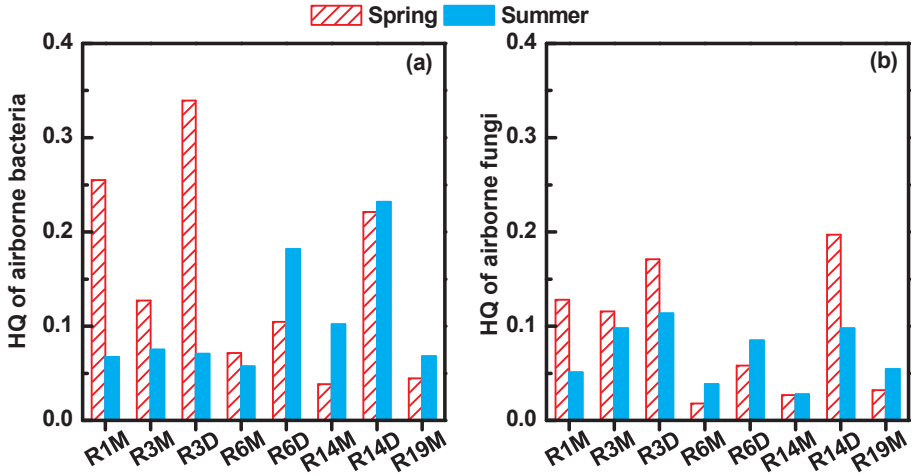

Fig. 6. The hazard quotient (HQ) of airborne bacteria (a) and fungi (b) by inhalation in spring and summer.

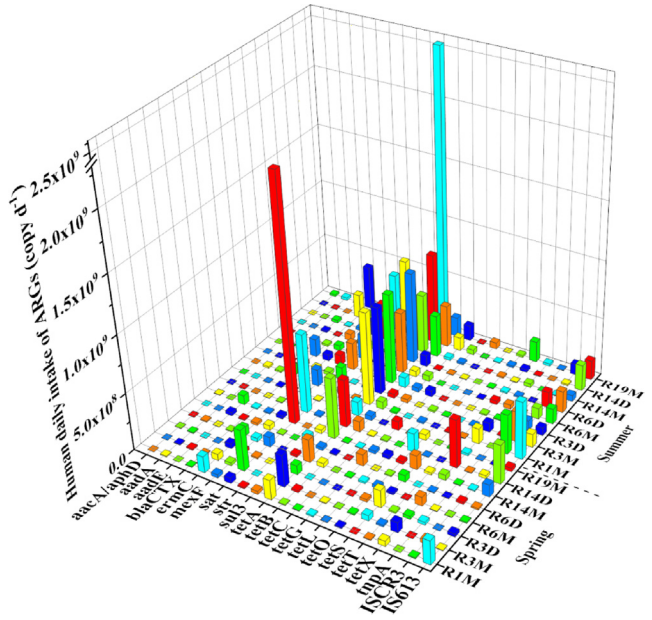

Fig. 7. The human daily intake of ARGs via inhalation of bioaerosols in spring and summer.

human being. Furthermore, what disconcerting us is that when we inhaled, the bacterial community in the respiratory tract could also be induced by the airborne ARGs to become ARB and further be expressed by human-borne bacteria, thus will cause more harm to respiratory system than that of those ARG-carrying dead cells and ARG itself (Lin et al., 2018). That means future studies should also focus on revealing whether and how airborne bacteria would interact with lung microbiomes and exchange with ARGs during inhalation.

4. Conclusions

In this study, the pollution profiles and correction of airborne pathogens and ARGs, as well as the associated health exposure risk were evaluated in detail, in Pearl River Estuaries in 2019. The results demonstrated that the levels of airborne pathogenic fungi and bacteria varied strongly with sampling sites and by the seasons, and the dominant proportion of bioaerosol was found in aerodynamic size < 4.7 μm. The diversity and abundance of bacterial community are higher than fungi with *Burkholderia-Paraburkholderia* and *Bacillales* as the two main dominant pathogens. Moreover, *tetA* and *tetB* were found to be the most abundant genes and the absolute abundance of ARGs in summer (1.1×10^8 copies m^{-3}) was also higher than in spring (5.7×10^7 copies m^{-3}). Pathogenic microbial community contributed significantly to the profiles of ARG of bioaerosol and *Escherichia-Shigella, Burkholderia-Paraburkholderia, Enterococcus, Clostridiaceae and Bacteroides* were the possible hosts of ARG. Inhalation risk of airborne microbes and ARGs were evaluated according to the human intake of environmental ARGs and opportunistic pathogens. Overall, the information provided in this study will be priceless especially during the outbreaks of fatal respiratory diseases and will be particularly important to control the spread of antibiotic-resistant respiratory pathogens.

CRediT authorship contribution statement

Zhishu Liang: Methodology, Writing - original draft. **Yun Yu:** Investigation. **Zikai Ye:** Data curation. **Guiying Li:** Writing - review & editing. **Wanjun Wang:** Validation. **Taicheng An:** Conceptualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105934.

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