Environmental Science & Technology

Article

Cite This: Environ. Sci. Technol. XXXX, XXX, XXX−XXX

pubs.acs.org/est

Global Survey of Antibiotic Resistance Genes in Air

Jing Li,[†] Junji Cao,[‡] Yong-guan Zhu,[§] Qing-lin Chen,[§] Fangxia Shen,[∥] Yan Wu,[⊥] Siyu Xu,[#] Hanqing Fan,[▽] Guillaume Da,[○] Ru-jin Huang,[‡] Jing Wang,[◆,¶] Alma Lorelei de Jesus,[∞] Lidia Morawska,[∞] Chak K. Chan,[⊗] Jordan Peccia,[⊙] and Maosheng Yao*[,†]

[†]State Key Joint Laboratory of Environmental Simulation and Pollution Control, College of Environmental Sciences and Engineering, Peking University, Beijing 100871, China

[‡]Key Lab of Aerosol Chemistry & Physics, Institute of Earth Environment, Chinese Academy of Sciences, Xi'an 710049, China

[§]Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China

[∥]School of Space and Environment, Beihang University, Beijing 100191, China

[⊥]School of Environmental Science and Engineering, Shandong University, Jinan 250100, China

[#]Department of Environmental Health Sciences, Graduate School of Public Health, Seoul National University, Seoul 08826, South Korea

[▽]Department of Earth and Environmental Engineering, Columbia University, New York, New York 10027, United States

[○]CERTES, Université Paris-Est Créteil, Centre d'études et de Recherche en Thermique, Environnement et Systèmes (CERTES), Créteil 94000, France

[◆]Institute of Environmental Engineering, ETH Zurich, Zurich 8093, Switzerland

[¶]Advanced Analytical Technologies, Empa, Dübendorf 8600, Switzerland

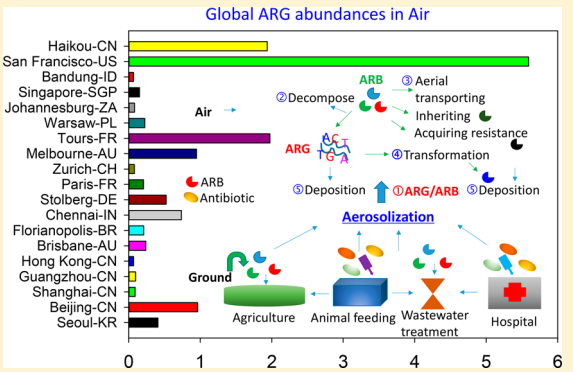
[∞]International Laboratory for Air Quality and Health, Queensland University of Technology, GPO Box 2434, Brisbane, Queensland 4001, Australia

[⊗]School of Energy and Environment, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong China

[⊙]Department of Chemical and Environmental Engineering, Yale University, New Haven, Connecticut 06520, United States

Supporting Information

ABSTRACT: Despite its emerging significant public health concern, the presence of antibiotic resistance genes (ARGs) in urban air has not received significant attention. Here, we profiled relative abundances (as a fraction, normalized by 16S rRNA gene) of 30 ARG subtypes resistant to seven common classes of antibiotics, which are quinolones, β-lactams, macrolides, tetracyclines, sulfonamides, aminoglycosides, and vancomycins, in ambient total particulate matter (PM) using a novel protocol across 19 world cities. In addition, their longitudinal changes in $PM_{2.5}$ samples in Xi'an, China as an example were also studied. Geographically, the ARGs were detected to vary by nearly 100-fold in their abundances, for example, from 0.07 (Bandung, Indonesia) to 5.6 (San Francisco, USA). The β-lactam resistance gene *blaTEM* was found to be most abundant, seconded by quinolone resistance gene *qepA*; and their corresponding relative abundances have increased by 178% and 26%, respectively, from 2004 to 2014 in Xi'an. Independent of cities, gene network analysis indicates that airborne ARGs were differentially contributed by bacterial taxa. Results here reveal that urban air is being polluted by ARGs, and different cities are challenged with varying health risks associated with airborne ARG exposure. This work highlights the threat of urban airborne transmission of ARGs and the need of redefining our current air quality standards in terms with public health.



INTRODUCTION

The world is currently facing a serious health threat resulting from both increasing antibiotic-resistant bacteria (ARB) infections and the multienvironmental spreading of antibiotic resistance genes (ARGs). For example, global mortality attributable to antimicrobial resistance (AMR) is estimated to

Received: April 25, 2018
Revised: June 26, 2018
Accepted: July 3, 2018

DOI: 10.1021/acs.est.8b02204
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

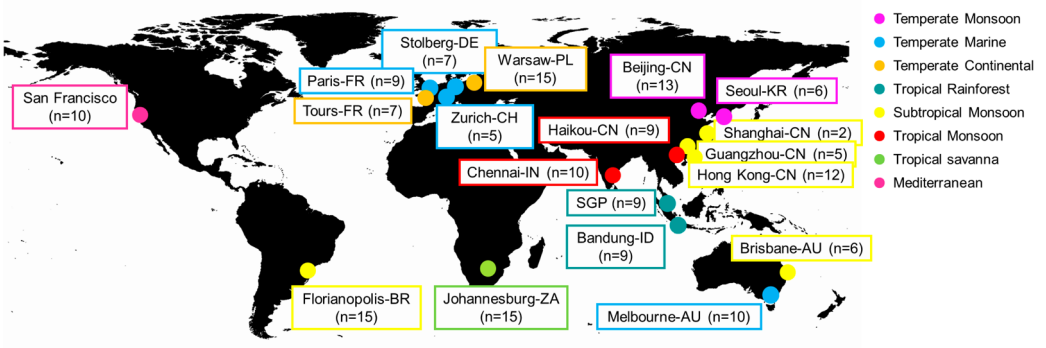


Figure 1. World map of automobile AC filter sample collection sites and the illustration of locations that represent different Köppen climate zones (the map was obtained using ArcGIS 10.2 (Esri); "n" in the map indicates the number of auto AC filters collected and analyzed for each city.

be near 700 000 per year, and is expected to rise to 10 million annually by 2050.[1,2] Major known contributors for AMR include animal husbandries, wastewater treatment plants (WWTPs), and hospitals.[3−8] In addition to their self-inheritance, ARGs can be acquired through horizontal gene transfer (HGT) from one bacteria to another; or from the environmental to human-related commensals and other bacteria with the assistance from mobile genetic elements (MGEs), such as plasmids, intergrons, transposes, and prophages.[5,9−15] Previous studies showed a strong correlation between ARGs and *intI1* (class 1 integron-integrase gene) in both natural and built environments,[14,15] which implies a potential for environmental AMR transfer and its impact on health.

Over the years, evidence for ARG presence and transmission in various environments is accumulating. For example, Levy et al. (1976) have earlier confirmed the spread of antibiotic-resistant plasmids in *Escherichia coli* from chicken to chicken and from chicken to human.[9] Zhu et al. (2017) investigated diverse and abundant ARGs in the sediments collected from 18 estuaries located 4000 km off the coast of China, and detected more than 200 ARG subtypes.[15] Airborne pathogens in hospital environments are frequently found to be multidrug-resistant,[4] which could pose a significant health risk to inhabitants even outside hospitals via airborne transmission.[16] Recently, there is an intense interest of studying the presence of ARGs and MGE genes in airborne PM in both occupational (e.g., hospitals, cattle feed yards, wastewater treatment plants, etc.) and urban environments.[8,17−23] Near concentrated animal feed operations, Ling et al. (2013) detected tetracycline resistance genes of *tetX* (100−200 copies/m^3) and *tetW* (100−400 copies/m^3) in human-occupied indoor environments in Colorado.[17] Li et al. (2016) reported relative abundances of *sul2* to sulfonamides and *intI1* in the air samples collected from a WWTP in Beijing.[19] Echeverria-Palencia et al. (2017) also detected airborne *blaSHA* (up to 10^2 copies/m^3) to *β*-lactams and *sul1* (up to 10^3 copies/m^3) in urban parks in California.[20] By reanalyzing the reported sequence data, Pal et al. (2016) indicated that bacterial biota in Beijing smog harbored a higher richness of 64.4 ARG types than other environments, for example, 38.9 for pharmaceutically polluted environments, 19.4 for wastewater/sludge, 11.8 for animals, 1.0−16.6 for humans and 1.6−3.3 for other terrestrial sources.[8] Hu et al. (2018) speculated that on highly polluted days the airborne particles might provide more adhesion sites which allow microbes to suspend more stably in the air.[22] These studies suggested that, in addition to other routes, airborne transmission may play an active role in environmental spread and exposure of antimicrobial resistance. Evidences show that such a risk varies with cities with differing antibiotic usages. For example, Pal et al. (2016) observed comparable relative abundances of ∼0.2 ARGs per 16S rRNA gene in ambient PM in Beijing, New York and San Diego, but about 5−8 times higher richness of ARG types detected in Beijing.[8] Using real-time qPCR technique, Echeverria-Palencia et al. (2017) found that the relative abundances of PM-borne ARGs in Los Angeles were approximately an order of magnitude lower than those in San Diego, Bakersfield and Fresno.[20] Hu et al. (2018) pointed out that in addition to different antibiotic usages, physicochemical factors, meteorological parameters and bacterial communities also affected the distribution patterns of PM-borne ARGs.[22] Unfortunately, such information on airborne ARGs for geographically, culturally, and economically disparate cities on a global scale is substantially lacking and their health impacts are unknown.

On the other hand, current air pollution health studies rely heavily on PM mass concentration, without considering biological parameters such as ARGs or ARB. In addition to long-range transport, varying levels of airborne ARGs in different cities with different antibiotic use patterns could create different exposure and transfer risks. The major objective of this study is to advance the knowledge of urban airborne ARGs through a novel global survey. We aim to document the spatial and temporal differences in the relative abundance profiles of ARGs in the air of different world cities, to determine the dominant ARG types, and to detail the airborne bacterial taxa that host the ARGs. Global information on the abundance and types of ARGs in ambient air is of great value in understanding current infectious disease transmission and providing valuable information for re-evaluating air quality assessment practice.

MATERIALS AND METHODS

Sample Collection and Pretreatment. To study the spatial variation of airborne ARGs on a global scale, we employed a previously established automobile air conditioning (AC) filter method[24] to obtain ambient total particulate matter (PM) samples from 19 cities situated in 13 countries across the globe under eight different climate zones (Figure 1). A total of 174 used AC filters were collected from randomly selected automobiles regardless of time or season from the year 2016 to 2017. Although there were some differences among the AC filters resulting from different automobile and/or filter brand, the filters will likely behave similar in terms with filtration efficiency as the PM accumulates over time. Here, the PM samples were first shaken from the AC filters onto a piece of white office paper, then poured into a 50 mL centrifuge tube

DOI: 10.1021/acs.est.8b02204
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

(Corning Premium Quality, Acton, MA), subsequently followed by a gravimetrical weight analysis using an analytical balance (AL204IC, Mettler Toledo, Inc., Greifensee, Switzerland). The tubes containing the PM samples from the AC filters were then extracted by corresponding needed volumes of sterile deionized (DI) water (Milli-Q, Millipore, Billerica, MA) to obtain the PM extraction samples which had a PM concentration of ∼1 mg/mL. The PM extraction solutions were further treated by vigorous vortexing for 15 min at a rate of 2800 rpm and then stored at −20 °C until subsequent experiments. As for the quality control, the same type of white office paper was used together with a new AC filter (purchased in Beijing, China) to perform the same procedures described above including dumping PM, pouring DI water and the extraction processes. The obtained 50 mL tube containing the DI water poured inside was used as a negative control.

We additionally investigated the relative abundance profiles of ARGs in $PM_{2.5}$ samples available for a 10-year time span in Xi'an, a northwestern city of China. The $PM_{2.5}$ samples were collected over the years at an urban monitoring site located on the rooftop (∼10 m above the ground) of the Chinese Academy of Sciences' Institute of Earth Environment building (E 108.887°, N 34.229°), surrounded by a residential area of ∼15 km south of downtown Xi'an. A portable atmospheric particulate matter sampler, MiniVol Tactical Air Sampler (TAS) (Airmetrics, Inc., Springfield, Oregon, USA), with a sampling flow rate of 5 L/min, was used to collect predefined 24-h $PM_{2.5}$ samples every day. $PM_{2.5}$ samples were collected onto quartz filters (47 mm, Whatman QM/A, England), which were presterilized by baking at 780 °C for 3 h prior to use. Each filter sample was sealed and stored at −20 °C until pretreatment for further analysis. A total of 72 quartz filter samples, respectively, from 6th or 7th, and 25th of each month (January to December) in 2004, 2009, and 2014 as listed in Supporting Information (SI) Excel file S1 were selected and analyzed. The $PM_{2.5}$ samples were extracted from quartz filters by the following procedure. First, a certain fraction (the analyzed deposition) (see SI Excel file S1) of each of the filters was cut out using a sterile cutter. Next, each filter fraction cut was placed together with 2 mL of sterile purified water (Milli-Q, Millipore, Billerica, MA) with 0.05% Tween 20 (Solarbio, Inc., Beijing, China) into in a sterile centrifuge tube (Corning Premium Quality, Acton, MA), then subjected to 20 min of sonication, and followed by vortex mixing (Vortex genie-2, Scientific Industries, Inc., NY) for 40 min at a speed of 2800 rpm. $PM_{2.5}$ extraction samples were stored at −20 °C until further experiments.

Detection of Antibiotic Resistance Genes (ARGs) in Air. In this work, a total of 39 ARG subtypes conferring resistance to 7 types of common antibiotics such as quinolones, β-lactams, macrolides, tetracyclines, sulfonamides, aminoglycosides, and vancomycins, and two mobile genetic element (MGE) genes including *tnpA* encoding transposase and *intI1* encoding integrase class I were screened by a high throughput real-time qPCR platform (Wcgene Biotech, Inc., Shanghai, China) in each city's PM extraction solution with a concentration of 1 mg/mL. For $PM_{2.5}$ samples collected in Xi'an, a total of 37 ARG subtypes resistant to β-lactams, quinolones, macrolides, sulfonamides, tetracyclines, and aminoglycosides, and 2 MGE genes of *tnpA* and *intI1* were screened. $PM_{2.5}$ extraction samples were respectively grouped by year (2004, 2009, 2014), and pooled together by season (Spring: March, April, and May; Summer: June, July, and August; Autumn: September, October, and November; and Winter: December, January, and February) for analysis. There were six samples in each group, as shown in SI Table S1. Samples from each group were pooled as a single sample with the same concentration. In doing so, we have obtained a total of 12 such mixed samples as listed in SI Table S1, respectively, representing the four seasons from the three mentioned years. Here, we analyzed the ARGs in Xi'an's ambient $PM_{2.5}$ samples and global ambient PM samples in two separate sequence batches with slightly different target ARG types.

DNA extractions of samples were performed according to the manufacture's guidelines as recommended by the SoilPure kit (DL127−01, Biomed, Inc., Beijing, China). The previously reported primer sets[7,25] as listed in SI Excel file S2 were used in this work for screening target resistance genes. The qPCR assay was carried out in a 10 μL volume containing 0.5 μL of template DNA, 5 μL of Roche FastStart Universal SYBR Green Master (ROX) (Hoffmann-La Roche, Inc., Basel, Switzerland), 3 μL of ddH_2O, 0.75 μL of each primer (10 μM each) using a ViiA7 real time PCR system (Applied Biosystems, Inc., Foster City, CA), with a threshold cycle (C_T) of 40 used as the detection limit. In addition to ARGs and MGE genes, the sequences of primers for 16s rRNA gene were as follows: forward primer: 5′-GGGTTGCGCTCGTTGC-3′, reverse primer: 5′-ATG-GYTGTCGTCAGCTCGTG-3′ (Wcgene Biotech, Inc., Shanghai, China). Cycling conditions were as follows: 95 °C for 10 min, followed by 40 cycles of (95 °C for 30 s and 60 °C for 30 s), last 72 °C for 10 min. The DI water from the control tube described above was used as a negative control for all qPCR assays and all their threshold cycles were under the detection limit. Here, we used the $2^{-\Delta CT}$ values[26] to compare relative abundances of ARGs between different samples:

$$\Delta C_T = C_{T,(ARG)} - C_{T,(16S\,rRNA)} \quad (1)$$

where $C_{T,(ARG)}$ and $C_{T,(16s\,rRNA)}$ are the threshold cycles, respectively, for ARG subtypes and 16s rRNA genes from typical qPCR experiments.

Bacterial 16S rRNA Gene Sequencing. For bacterial community analysis, a total of 19 samples for all cities' ∼1 mg/mL of PM extraction solution from automobile AC filters as well as all of the 72 Xi'an $PM_{2.5}$ extraction samples were studied by a 16S rDNA bacterial amplicon sequencing by the Illumina Miseq 2 × 300 bp sequencing platform (Sangon Biotech, Inc., Shanghai, China) with a sequencing depth of 20, 000 reads per sample. The V3−V4 region of 16S rRNA gene was chosen to amplify with the forward primer 341F (CCTACGGGNGGCWGCAG) and the reverse primer 805R (GACTACHVGGGTATCTAATCC). Details on sample preparation and sequencing were also provided in SI Section S1.

Statistical Analysis. Heat maps for relative abundance profiles based on the $2^{-\Delta CT}$ values of ARGs were generated by HemI 1.0 according to a previous reference.[27] Network analysis based on Spearman's rank correlations was used to visualize the co-occurrence of airborne subtypes internally and with bacterial communities, and analyzed in the R software environment according to the method described previously.[28] Network visualization was conducted using the interactive platform of Gephi[29] with Fruchterman Reingold placement algorithm.[30] One Way ANOVA and Paired *t* test were respectively performed using Sigma Plot 12.5 (Systat Software, Inc., Chicago, IL) to compare the relative abundances of ARGs among different seasons and those between 2004 and 2014. Wilcoxon signed rank test was used using Sigma Plot 12.5 to compare the $PM_{2.5}$ concentrations between 2004 and 2014 because the correspond-

DOI: 10.1021/acs.est.8b02204
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

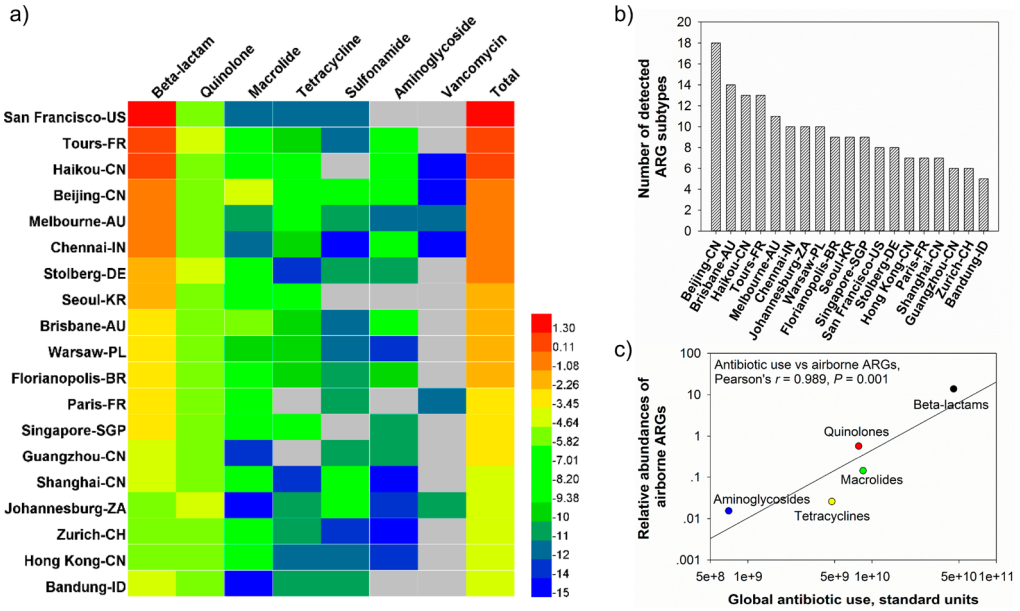


Figure 2. (a) The relative abundance profiles of ARGs in 7 antibiotic types and (b) numbers of detected airborne ARG subtypes across 19 different world cities; and (c) the correlation of the relative abundances of airborne ARG types in the present study with the global antibiotic consumption in 2010 reported previously[35] in five common antibiotic types. Values plotted in (b) are logarithms (to the base 2) of the corresponding relative abundances; gray squares in (a) represent those with ARG concentrations below the detection limit from qPCR.

ing data were not Gaussian-distributed. Alpha diversity was calculated in Mothur 1.30.1 according to the literature by Schloss et al. (2009)[31] for bacterial community richness (Ace and Chao) and diversity (Shannon and Simpson) based on a similarity confidence level of 97%. Nonmetric Multi-Dimensional Scaling (NMDS) based on Bray−Curtis distance was used to compare bacterial community structures among samples. Analysis of Similarity (ANOSIM) was used to compare the variations in bacterial community structures among two or more PM sample groups statistically. NMDS and ANOSIM were performed by R software (version 3.2) with the vegan package 2.0−10. A p-value of 0.05 indicates a statistically significant difference in all statistical analyses.

RESULTS AND DISCUSSION

Spatial Variations of Airborne ARGs and MGE Genes Observed Across Different Geophysical Locations. In this work, a total of 39 ARG subtypes were analyzed, but only 30 ARG subtypes were detected in airborne particulate matter collected from 19 global cities as marked in Figure 1. Figure 2 a) shows relative abundances of ARG subtypes grouped into the seven primary antibiotic types and ARG subtype richness in ambient PM for different cities. Beijing was found to have the highest richness (up to 18 subtypes) of airborne ARGs, accounting for 60% of total detected ARGs; while the ambient PM in Bandung was found to contain only 5 subtypes of ARGs (Figure 2 b)). As shown in Figure 2a), the genes providing resistance to β-lactams (0.03−5.6, normalized by 16S rRNA gene, hereafter) and quinolones (0.02−0.05) were the top two most abundant types of ARGs in all 19 cities, followed by macrolide (5.2×10^{-5}−5.1×10^{-2}), tetracycline (1.9×10^{-4}−5.2×10^{-3}), sulfonamide (4.2×10^{-5}−9.1×10^{-3}), aminoglycoside (2.9×10^{-5}−2.9×10^{-3}), and vancomycin (2.5×10^{-5}−7.1×10^{-4}). These results agreed with a previous independent study which showed that β-lactam resistance genes also predominated in Beijing smog.[8] The relative abundance of β-lactam resistance genes detected here varied by over 2 orders of magnitude across different cities, with the highest abundance observed for San Francisco (Figure 2 b)). In contrast, Johannesburg (0.03), Zurich (0.04), and Hong Kong (0.04) had the lowest relative abundances of airborne β-lactam resistance genes. If all detected types of resistance genes considered, airborne ARGs were found to be the most abundant for San Francisco (5.6) and the least for Bandung (0.07) as observed in Figure 2 a). The detected airborne ARGs could be due to the direct emission of ARGs-carrying bacteria or their reaerosolization due to natural winds or various ground human activities from urban city environments.

The above ARG results reflect previously reported resistance patterns of isolated pathogen strains in hospitals. For example, early in 1996−1999, resistance rates to ceftazidime (cephalosporins), which can be used as the proxy for inducible and extended-spectrum-β-lactam, of *Enterococcus cloacae* and *Pseudomonas aeruginosa* strains in San Francisco County, were at 39% and 13%, respectively.[32] In a 2015 inpatient data set from 346 U.S. hospitals, the nonsusceptible rates of *Pseudomonas aeruginosa* to ciprofloxacin/levofloxacin (quinolones), ceftazidime (cephalosporins, hereinafter), cefepime, meropenem, and piperacillin/tazobactam were, respectively, up to 33.6%, 19.4%, 19.8%, 20.9%, and 14.0%.[33] As such, in 2012 in the U.S., an order for limiting the use of cephalosporin was enacted into law.[34] It is reasonable to conclude that the antibiotic use patterns for different antibiotic types are responsible for the variations in the relative abundance distribution of airborne ARG types. Through reanalyzing the existing global antibiotic drug consumption data by five major common types (no detailed data for their subtypes) including β-lactams, quinolones, macrolides, tetracyclines, and aminoglycosides,[35] we found a positive linear correlation (Pearsons' r = 0.989, p-value = 0.001) between the global relative abundances of airborne ARGs detected and the above five antibiotic drug hospital consumption data as shown in Figure 2 c). This ARG relationship with human consumption stems from the fact that the global PM was collected via automobile AC filter mainly inside the cities where other ARG

DOI: 10.1021/acs.est.8b02204
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

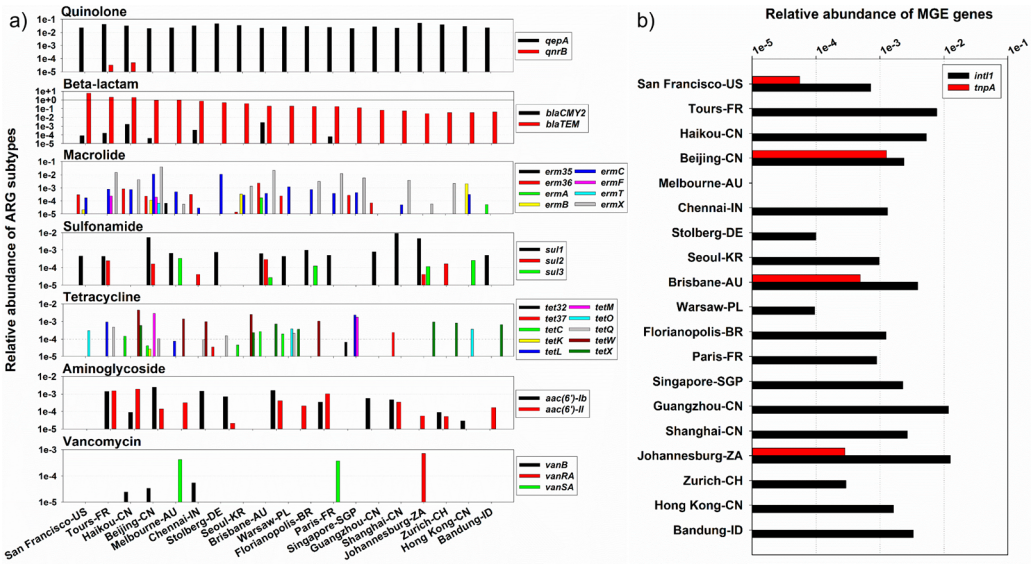


Figure 3. Grouped bar plots of (a) detected ARG subtypes for seven antibiotics (Quinolone, Beta-lactam, Macrolide, Sulfonamide, Tetracycline, Aminoglycoside, and Vancomycin); and (b) detected mobile genetic element (MGE) genes across 19 world cities; data are plotted in relative abundances based on $2^{-\Delta CT}$ values.

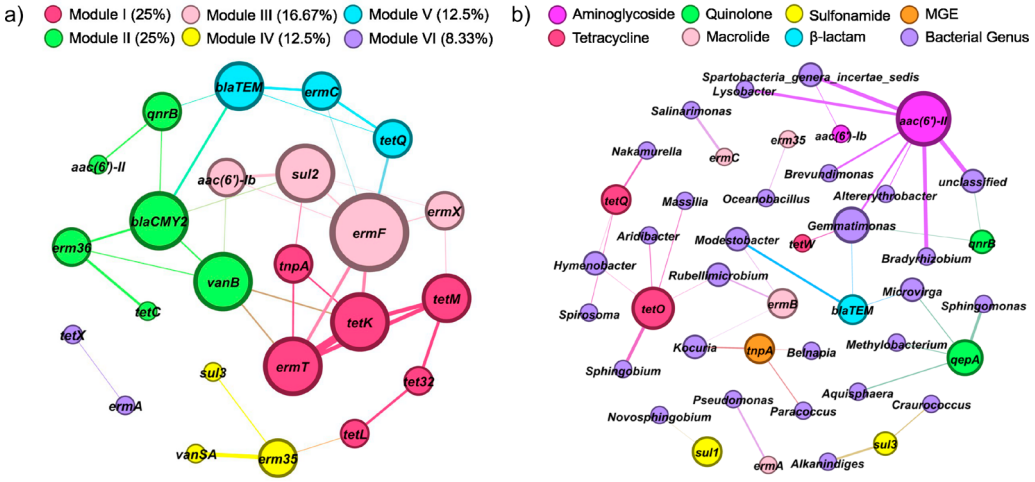


Figure 4. Co-occurrence and contributor network of airborne ARG subtypes in PM samples: (a) between ARGs and (b) associations between ARGs and the most relatively abundant 50 bacterial genera detected using gene sequence in one batch across 19 world cities as depicted in Figure 1.

emission sources such as agriculture lands, wastewater treatment plants and also animal feeding houses are fewer or located in the suburbs. Accordingly, different practices for controlling the antibiotic use for urban hospitals could significantly affect the emissions of ARGs into the air, thus consequently on the health and environment in an urban city.

The relative abundances of ARGs divided into 30 subtypes and two MGE genes such as TnA genes and intI genes for each of the 19 cities are shown in Figure 3a) and Figure 3b), respectively. As observed in Figure 3a), *qepA* (ciprofloxacin, etc.) and *blaTEM* (cephalosporins especially in *β*-lactams, such as ceftazidime, cefepime, etc.) were found to dominate the ARGs detected for all cities. Such results are expected as genes of *blaTEM* and *qepA* have been previously observed to be widespread and abundant across various external environments such as sediment, water, soil, mine, wastewater/sludge, pharmaceutical pollution, and high pollution atmosphere.[8,36] In comparison with natural environments, macrolide and tetracycline resistance genes are more abundant in the human microbiome.[8] However, the distribution patterns of airborne macrolide and tetracycline resistance genes varied greatly with cities as observed in Figure 3a), and did not correlate with the population. This observation was obviously expected as people's drug use preference, health conditions and so on can differ for different cities. The relative abundances of sulfonamide resistance genes in Shanghai (9.1×10^{-3}), Beijing (2.8×10^{-3}) and Johannesburg (1.6×10^{-3}) were found to be 1−2 orders of magnitude higher than those in other cities (4.2×10^{-5}-7.8×10^{-4}). The results indicate that ARG emissions from certain occupational environments such as wastewater treatment and pharmaceutical plants in or near these cities deserve a particular attention since sulfonamide resistance genes were much more frequently detected in these places.[7,8] It is certainly alarming that ARGs providing resistance to vancomycins, though at low levels (2.5×10^{-5}−7.1×10^{-4}), were also detected in PM samples collected from Beijing, Haikou, Chennai, Melbourne, Paris, and Johannesburg. Vancomycin has so far been considered to be the most powerful antibiotics

DOI: 10.1021/acs.est.8b02204
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

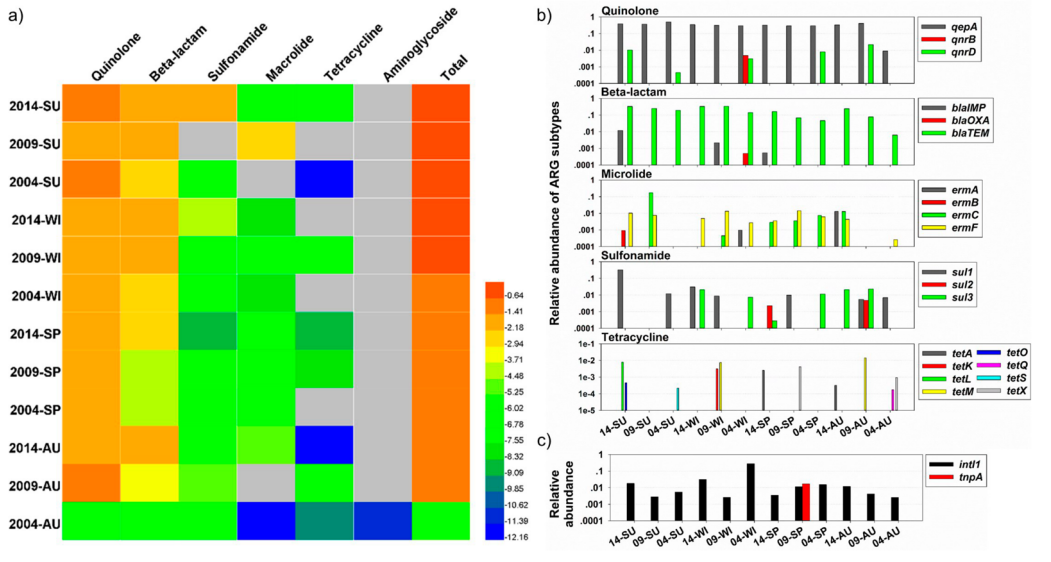


Figure 5. Abundance profiles of (a) ARGs in 6 types as well as their total subtypes, (b) the grouped bar plots of detected ARG subtypes in 6 types, and (c) detected mobile genetic element (MGE) genes in $PM_{2.5}$ samples collected in different seasons among the years of 2004, 2009, and 2014; Values plotted in (a) are logarithms (to the base 2) of the corresponding relative abundances; gray squares in (a) represent concentrations of the corresponding ARGs were lower than detection limit of qPCR; (b) and (c) are plotted in relative abundances based on $2^{-\Delta CT}$ values.

and is the last resort to clear methicillin-resistant *Staphylococcus* aureus (MRSA) that is causing 80−90% of hospital-acquired infection cases and emerging to resist nearly all current common antibiotic classes.[3,37−40]

On another front, MGEs were considered to contribute significantly to the evolution and proliferation of multiple antibiotic-resistant bacteria.[5,9−15] Here, both airborne *intI1* and *tnpA* encoding MGEs were detected in 18 of 19 cities studied, with an exception for Melbourne as shown in Figure 3b). Expectedly, airborne *intI1* was found to be more abundant (9.52×10^{-5}−1.25×10^{-2} across 18 of 19 cities) than *tnpA* (5.54×10^{-5}−1.26×10^{-3} across 4 of 19 cities). *IntI1*, the class 1 integron-integrase gene, is the most widespread and abundant MGE gene detected across various environments including human microbiome, animal-associated environments, sediment, water, soil, mine, wastewater/sludge, pharmaceutical, and ambient PM pollutants.[8] One recent study indicated that *IntI1* might play a diluting role in horizontal gene transfer across the land use gradients, for example, from rural to urban and industry.[23] Our results indicate that airborne PM is carrying the ARGs that are emitted from various environments, and further transported to other places through the atmospheric movement. The potential integration and subsequent expression of these ARGs in different receptors such as humans and ecology lands can have serious societal consequences. For example, these ARBs could be subsequently dispersed among people as indicated in a previous study.[41] The observed different levels of airborne ARG subtypes and MGEs as shown in Figure 3 implied different spreading potentials of airborne ARGs in different cities, accordingly representing different exposure risks for health and ecology.

Bacterial Genera and ARG Co-Occurrences Regardless of Geophysical Locations. In this work, we have also analyzed the bacterial communities associated with the airborne ARGs detected for different cities. Figure 4 shows the co-occurrence network of airborne ARG subtypes (a) and associations between ARG subtypes and 50 most abundant bacterial genera (b) based on statistically significant correlations (Spearman's rank correlation analysis, p-value < 0.05). The relative abundances of bacterial communities at the genus level for each city are presented in SI Figure S1. The size of each node in Figure 4 is proportional to the number of connections, and the thickness of each edge (connection) between two nodes is proportional to the value of Spearman's rank correlation coefficients (r) according to a previous reference.[42] As seen in Figure 4a), there are 24 nodes and 37 edges with Spearman's r-values ranging from 0.46 to 1.00, and all associations presented here are statistically significant (p-values < 0.05). According to Newman (2006),[43] the modularity calculated by Gephi-0.9.2 is 0.441, suggesting that the network could be classified into 6 modules. As shown in Figure 4, both Module I and II were the largest modules totally accounting for 50% of the whole co-occurrence pattern while Module III accounted for 16.67% of the ARG co-occurrence. The genes of *blaCMY2*, *ermT*/*tetK*, and *ermF* were the respective hubs for the Modules I, II, and III. The hubs detected here show other related co-occurring ARG subtypes in each module that share the same bacterial communities as their likely hosts.[28] For example, the hub of *ermF* encoding resistance to macrolides in the Module III can be the indicator of the genes of *ermX* (macrolides), *sul2* (sulfonamides) and *aac(6′)-Ib* (aminoglycosides) in airborne PM. The results here suggest that these four genes may be statistically associated with the same host bacteria across different cities, and thus possibly can be used as indicators for the airborne 24 ARG subtypes detected.

The detected ARGs were found to be associated differentially with bacterial taxa as demonstrated in the network analysis in Figure 4. Figure 4b) shows 43 nodes and 38 edges with Spearman's r-values ranging from 0.46 to 0.64, and all associations presented in Figure 4a) are statistically significant (p-values < 0.05). Data in Figure 4a) reveal potential host information for the ARGs in complex airborne PM. This also means that the ARGs and their host bacterial taxa might share similar trends on the relative abundances among different cities.[22,28,44] As shown in Figure 4b, the gene of *aac(6′)-II* encoding resistance to aminoglycosides was found to have significant associations with seven bacterial genera including

DOI: 10.1021/acs.est.8b02204
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

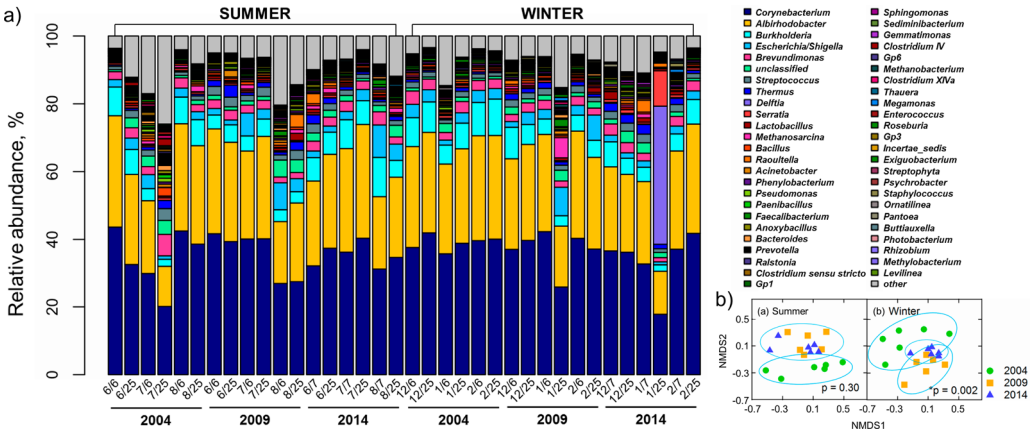


Figure 6. (a) Distribution bar plot of $PM_{2.5}$-borne bacterial community compositions in $PM_{2.5}$ samples collected in summer and winter on the genus level and the corresponding illustration of each genus, with sampling dates displayed in *X*-axis; and (b) NMDS analyses for the relative abundances of $PM_{2.5}$-borne bacterial genera in respective summer and winter among the years of 2004, 2009, and 2014 over a 10-year time span, where *p* is the statistical result of ANOSIM; **p*-value < 0.05 indicates a statistical significance.

Brevundimonas, *Gemmatimonas*, *Spartobacteria*, etc. The genes of *tetO* (tetracyclines) and *qepA* (quinolones) were also associated with five and four different bacterial genera, respectively. It is interesting to note that the MGE gene of *tnpA* was found to be associated with *Kocuria*, *Belnapia* and *Paracoccus* in the airborne microbiota. Among others, the *Gemmatimonas* genus was shown to be associated with the most ARG subtypes including the most dominant ARG of *blaTEM* as well as *aac(6′)-II*, *qnrB* and *tetW*, while *Microvirga* was significantly associated with both the two dominant ARGs, *blaTEM* and *qepA*. Unlike *Gemmatimonas* and *Microvirga*, the *Spartobacteria* genus was shown to be predominantly associated with aminoglycoside resistance genes as it was only significantly associated with the genes of *aac(6′)-II* and *aac(6′)-Ib*. On the other hand, the *Hymenobacter* genus was shown to be predominantly associated with tetracycline resistance genes. Particularly, the dominant bacterial genus in air, *Pseudomonas*, consisting human opportunistic species, was detected to be significantly associated with the macrolide resistance gene of *ermA*, suggesting *Pseudomonas* might contribute to the dissemination of *ermA* gene. These results collectively imply that airborne PM containing different bacterial community structures from different cities could impose significantly different AMR exposure health risks.

Significant Temporal Variations Detected for $PM_{2.5}$-borne ARGs and MGEs over the Past Decade. To further examine the longitudinal dynamics of airborne AMR, we investigated whether such a risk has increased over the past years. As an example, we studied a total of 37 ARG subtypes in Xi'an, but only detected 22 ARG subtypes in $PM_{2.5}$ samples that are available for analysis from 2004, 2009, and 2014. Figure 5a) indicates the patterns and degrees of enrichments of ARGs in 6 types as well as in the total ARGs across different seasons in 2004, 2009, and 2014 in Xi'an. In general, the relative abundances of $PM_{2.5}$-borne ARGs in summer (0.86 ± 0.20, average ± standard deviation, hereafter) were the highest among all seasons (*p*-value = 0.023, One Way ANOVA), followed by winter (0.63 ± 0.15), spring (0.42 ± 0.07) and fall (0.40 ± 0.33). It was observed here that from the year 2004 to 2014, the relative abundances of total ARGs in $PM_{2.5}$ increased in all seasons (by 57% in summer, 60% in winter, 32% in spring, and 2,486% in autumn). Statistical analysis shows that the relative abundances of $PM_{2.5}$-borne ARGs in 2014 were significantly higher than those a decade ago in Xi'an (*p*-value = 0.041, Paired *t* test). However, there was no significant difference detected in the $PM_{2.5}$ concentrations between 2004 and 2014 (*p*-value = 0.157, Wilcoxon signed rank test), implying that the differences are taking place in ground-based human activities that involve use of antibiotics.

The relative abundances of ARG subtypes and spreading potentials were also detected to have evolved, for example, in Xi'an, in this work. The ARG subtypes classified into six types and MGE genes were respectively shown in Figure 5b) and Figure 5c) for different seasons of 2004, 2009, and 2014. The ARGs of *qepA* (0.322 ± 0.144) and *blaTEM* (0.183 ± 0.118), respectively, providing resistance to quinolones and *β*-lactams were found to be the top two abundant ARG subtypes over the past decade in Xi'an (Figure 5b). From the year 2004 to 2014, the relative abundances of *qepA* and *blaTEM* had respectively increased by 26% (*p*-value = 0.483, Paired *t* test) and 178% (*p*-value = 0.008, Paired *t* test). This observation corresponds well with the findings in Sun and Wang (2012) that the daily defined doses (DDDs) of the levofloxacin (quinolone) and cephalosporins (*β*-lactam) were reported to have respectively increased by 109% and 427% from the year 2008 to 2010 in Xi'an Huashan Central Hospital.[45] The gene of *blaTEM*, typically encoding resistance to cephalosporins, was recently found to be prevalent in carbapenem-resistant *Acinetobacter baumannii* isolates (33.33%) and third generation cephalosporins-resistant *Enterobacteriaceae* including *Acinetobacter* spp. (25.9%) and *Klebsiella* spp. (30.6%).[46−48] These species are all critical priority pathogens according to the World Health Organization (WHO) global priority pathogens list.[49] Interestingly, the MGE genes of *intI1* were detected in all $PM_{2.5}$ samples, while *tnpA* was only found for the spring of 2009 as shown in Figure 5c). Statistically, our study suggests that during the past decade, the abundances of urban ambient $PM_{2.5}$-borne ARGs have increased due to changes from the ground human activities, for example, hospital usages of antibiotics. Increases in abundances of airborne ARGs inevitably could lead to increased "second-hand" inhalation risk of ARGs for city inhabitants. Different from the chemical products, ARGs could transmit among people as well as bacterial species, thus increasing the susceptibility of humans or the environment to bacterial resistance. The results from Xi'an here also indicate that such a threat has actually increased over the past decade.

DOI: 10.1021/acs.est.8b02204
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Temporal Variations of $PM_{2.5}$-Borne Bacterial Community during the Past Decade: Potential Impacts from AMRs. As an example for potential influences of ARGs, we have investigated the changes in $PM_{2.5}$-borne bacterial community structures in Xi'an. We sequenced all the $PM_{2.5}$ samples in two batches, respectively, the group from summer grouped with winter, and that from spring grouped with autumn; however, there was an unidentified error for the sequencing process regarding the group of spring and autumn samples. Thus, here we only show the results for the groups of summer and winter $PM_{2.5}$ samples. Phylogentic compositions of $PM_{2.5}$-borne bacteria at the genus level and corresponding nonmetric multidimensional scaling (NMDS) analyses for summer and winter among 2004, 2009, and 2014 were provided and analyzed as shown in Figure 6a) and Figure 6b), respectively. The statistics reflecting alpha diversity such as Ace, Chao, Shannon, and Simpson were listed in SI Excel file S3. Interestingly, we have detected significant differences in the airborne bacterial genus structures for winter seasons for 2004, 2009, and 2014 (ANOSIM, hereinafter, R = 0.2, p-value = 0.002) as shown in Figure 6 b); whereas the interdecadal change in bacterial genus structures for summer was not statistically significant as seen in Figure 6 b) (p-value = 0.3). In winter, both $PM_{2.5}$-borne bacterial community richness and diversity had increased during the past decade (SI Excel file S3). For both winter and summer, the bacterial structures for 2004 and 2009 were statistically separated as seen in Figure 6 b), implying that significant differences have taken place with respect to bacterial emissions from the ground from the year 2004 to 2009. For genus level, compared to earlier years, the average relative abundances of *Faecalibacterium* and *Methanosarcina* in the year 2009, and those of *Delftia*, *Serratia*, and *Raoultella* in the year 2014 had significantly increased in winter (Figure 6a)). Most of these bacterial genera are facultatively anaerobic or strictly anaerobic, which are often enriched in anthropogenic sources such as guts of human and animals, composting, biogas, and wastewater treatment plants according to the literature.[50−54] Therefore, the changes in winter might be explained by the increasing contribution from anthropogenic sources to airborne $PM_{2.5}$ that resulted from the increasing population density (from 717 person/km^2 in the year 2004 to 807 person/km^2 in the year 2014 according to Xi'an Statistical Yearbooks, 2005−2015)[55] over the past decade. Nonetheless, for summer, bacterial community compositions in the year 2014 were clearly different from those for both the year 2004 and 2009 as shown in Figure 6b). Unexpectedly, Alpha diversity statistics (SI Excel file S3) implied that for summer with more favorable bacterial growth conditions such as temperature and humidity compared to other seasons (see the meteorological parameters of different months in Xi'an in the SI Table S2 and S3), there was a diversity loss detected in airborne bacterial biota. The changes in airborne ARGs directly reflected those occurring from the ground. Our results suggest that different bacterial emissions from the ground were taking place during the winner, for example, an increase in hospital usages of antibiotics due to increasing population, thus resulting in changes in airborne ARGs. The use of antibiotics can eliminate or diminish certain bacteria by promoting abundances of antibiotic-resistant genes and bacteria, and thus inhibiting the growth of other competing bacteria.[56−58] Previous studies have also demonstrated the loss of bacterial community diversity in various environmental media (e.g., human airways, effluents and soils from animal husbandry facilities) that are frequently subjected to a variety of antibiotics.[56,59−61] As an emerging biological pollutant, airborne ARGs when inhaled could likewise induce the disequilibrium of respiratory tract bacterial community and thus affect the immune system if up-taken and further expressed by human-borne bacteria. Due to aerial transport, remote regions even without using antibiotics could be exposed to the "second hand" ARGs, which are initially being developed in other regions but transported elsewhere. Results here imply that urban air is being polluted by ARGs, and different regions are challenged with varying but increasing respiratory health risks related to PM-borne ARGs or ARB. Among the detected cells in urban air, those airborne viable bacteria carrying different ARGs can certainly cause more harm than ARG itself and those ARG-carrying dead cells. However, the long-term microecological consequences for both atmosphere and human respiratory system resulting from exposure of airborne ARGs remain to be further explored. In future research endeavors, more cities or regions on the global scale can be also included for both spatial and temporal pattern analyses of airborne ARGs. This work highlights the threat of airborne transmission of ARGs and the need of redefining our current air quality standards in terms with public health in an urban city.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b02204.

Experimental protocols of sample preparation and sequencing; $PM_{2.5}$ sample information from Xi'an; meteorological data for Xi'an ; airborne bacterial community compositions in 19 worldwide cities on the genus level; The statistics reflecting alpha diversity, including Ace, Chao, Shannon, and Simpson for $PM_{2.5}$-borne microbiota and abundances of bacterial genera; The PCR primer sets used in this work for screening antibiotic resistance genes (PDF)

The collection and usage information of total $PM_{2.5}$ samples collected in Xi'an, China (XLSX)

The primer sets used in this work for screening antibiotic resistance genes (XLSX)

The statistics reflecting alpha diversity, including Ace, Chao, Shannon, and Simpson for $PM_{2.5}$-borne microbiota during winter and summer, from 2004 to 2014, in Xi'an, China (XLSX)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: Yao@pku.edu.cn.

ORCID

Lidia Morawska: 0000-0002-0594-9683

Maosheng Yao: 0000-0002-1442-8054

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was supported by the NSF of China Distinguished Young Scholars Fund Awarded to M. Yao (21725701), and the National Natural Science Foundation of China (91543126, 21611130103, 21477003, 41121004), the Ministry of Science and Technology (2016YFC0207102, 2015CB553401, and 2015DFG92040).

DOI: 10.1021/acs.est.8b02204
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

REFERENCES

(1) *Review on Antimicrobial Resistance. Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations*; The Wellcome Trust, 2014.

(2) *Review on Antimicrobial Resistance. The Review on Antimicrobial Resistance, Tackling Drug-Resistant Infections Globally: Final Report and Recommendations*; The Wellcome Trust, 2016.

(3) Pruden, A.; Pei, R. T.; Stortboom, H.; Carlson, K. H. Antibiotic resistance genes as emerging contaminants: Studies in Northern Colorado. *Environ. Sci. Technol.* **2006**, *40*, 7445−7450.

(4) Dijkshoorn, L.; Nemec, A.; Seifert, H. An increasing threat in hospitals: Multidrug-resistant Acinetobacter baumannii. *Nat. Rev. Microbiol.* **2007**, *5*, 939.

(5) Gilchrist, M. J.; Greko, C.; Wallinga, D. B.; Beran, G. W.; Riley, D. G.; Thorne, P. S. The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. *Environ. Health. Persp.* **2007**, *115*, 313−316.

(6) Zhu, Y. G.; Johnson, T. A.; Su, J. Q.; Qiao, M.; Guo, G. X.; Stedtfeld, R. D.; Hashsham, S. A.; Tiedje, J. M. Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 93435−93440.

(7) Yang, Y.; Li, B.; Ju, F.; Zhang, T. Exploring variation of antibiotic resistance genes in activated sludge over a four-year period through a metagenomic approach. *Environ. Sci. Technol.* **2013**, *47*, 10197−10205.

(8) Pal, C.; Bengtssonpalme, J.; Kristiansson, E.; Larsson, D. G. J. The structure and diversity of human, animal and environmental resistomes. *Microbiome* **2016**, *4*, 54.

(9) Levy, S. B.; Fitzgerald, G. B.; Macone, A. B. Spread of antibiotic resistance plasmids from chicken to chicken and from chicken to man. *Nature* **1976**, *260*, 40−42.

(10) Kruse, H.; Sorum, H. Transfer of multiple-drug resistance plasmids between bacteria of diverse origins in natural microenvironments. *Appl. Environ. Microbiol.* **1994**, *60*, 4015−4021.

(11) Heuer, H.; Smalla, K. Plasmids foster diversification and adaptation of bacterial populations in soil. *FEMS Microbiol. Rev.* **2012**, *36*, 1083−1104.

(12) Gillings, M. R.; Gaze, W. H.; Pruden, A.; Smalla, K.; Tiedje, J. M.; Zhu, Y. G. Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. *ISME J.* **2015**, *9*, 1269−1279.

(13) Martinez, J. L.; Coque, T. M.; Baquero, F. What is a resistance gene? Ranking risk in resistomes. *Nat. Rev. Microbiol.* **2015**, *13*, 116−123.

(14) Ma, L.; Li, A. D.; Yin, X. L.; Zhang, T. The Prevalence of Integrons as the carrier of antibiotic resistance genes in natural and man-made environments. *Environ. Sci. Technol.* **2017**, *51*, 5721−5728.

(15) Zhu, Y. G.; Zhao, Y.; Li, B.; Huang, C. L.; Zhang, S. Y.; Yu, S.; Chen, Y. S.; Zhang, T.; Gillings, M. R.; Su, J. Q. Continental-scale pollution of estuaries with antibiotic resistance genes. *Nat. Microbiol.* **2017**, *2*, 16270.

(16) Lis, D. O.; Pacha, J. Z.; Idzik, D. Methicillin resistance of airborne *Coagulase-negative Staphylococci* in homes of persons having contact with a hospital environment. *Am. J. Infect. Control* **2009**, *37*, 177−182.

(17) Ling, A.; Pace, N. R.; Hernandez, M. T.; Lapara, T. M. Tetracycline resistance and class 1 integron genes associated with indoor and outdoor aerosols. *Environ. Sci. Technol.* **2013**, *47*, 4046−4052.

(18) McEachran, A. D.; Blackwell, B. R.; Hanson, J. D.; Wooten, K. J.; Mayer, G. D.; Cox, S. B.; Smith, P. N. Antibiotics, bacteria, and antibiotic resistance genes, Aerial transport from cattle feed yards via particulate matter. *Environ. Health. Persp.* **2015**, *123*, 337−343.

(19) Li, J.; Zhou, L. T.; Zhang, X. Y.; Xu, C. J.; Dong, L. M.; Yao, M. S. Bioaerosol emissions and detection of airborne antibiotic resistance genes from a wastewater treatment plant. *Atmos. Environ.* **2016**, *124*, 404−412.

(20) Echeverria-palencia, C. M.; Thulsiraj, V.; Tran, N.; Ericksen, C. A.; Melendez, I.; Sanchez, M. G.; Walpert, D.; Yuan, T.; Elizabeth, F.; Senthilkumar, N.; Sun, F.; Li, R.; Hernandez-Cira, M.; Gamboa, D.; Haro, H.; Paulson, S. E.; Zhu, Y.; Jay, J. A. Disparate antibiotic resistance gene quantities revealed across 4 major cities in California: A survey in drinking water, air, and soil at 24 public parks. *ACS Omega* **2017**, *2*, 2255−2263.

(21) Gao, M.; Jia, R.; Qiu, T.; Han, M.; Wang, X. Size-related bacterial diversity and tetracycline resistance gene abundance in the air of concentrated poultry feeding operations. *Environ. Pollut.* **2017**, *220*, 1342−1348.

(22) Hu, J.; Zhao, F.; Zhang, X. X.; Li, K.; Li, C.; Ye, L.; Li, M. Metagenomic profiling of ARGs in airborne particulate matters during a severe smog event. *Sci. Total Environ.* **2018**, *615*, 1332−1340.

(23) Xie, J.; Jin, L.; Luo, X.; Zhao, Z.; Li, X. 2018. Seasonal disparities in airborne bacteria and associated antibiotic resistance genes in PM2. 5 between urban and rural sites. *Environ. Sci. Technol. Lett.* **2018**, *5*, 74−79.

(24) Li, J.; Li, M. Z.; Shen, F. X.; Zou, Z. L.; Yao, M. S.; Wu, C. Y. Characterization of biological aerosol exposure risks from automobile air conditioning system. *Environ. Sci. Technol.* **2013**, *47*, 10660−10666.

(25) Looft, T.; Johnson, T. A.; Allen, H. K.; Bayles, D. O.; Alt, D. P.; Stedtfeld, R. D.; Sul, W. J.; Stedtfeld, T. M.; Chai, B.; Cole, J. R.; Hashsham, S. A.; Tiedje, J. M.; Stanton, T. B. In-feed antibiotic effects on the swine intestinal microbiome. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 1691−1696.

(26) Schmittgen, T. D.; Livak, K. J. Analyzing real-time PCR data by the comparative CT method. *Nat. Protoc.* **2008**, *3*, 1101−1108.

(27) Deng, W.; Wang, Y.; Liu, Z.; Cheng, H.; Xue, Y. HemI: A Toolkit for Illustrating Heatmaps. *PLoS One* **2014**, *9*, e111988.

(28) Li, B.; Yang, Y.; Ma, L.; Ju, F.; Guo, F.; Tiedje, J. M.; Zhang, T. Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. *ISME J.* **2015**, *9*, 2490−2502.

(29) Bastian, M.; Heymann, S.; Jacomy, M. Gephi: An open source software for exploring and manipulating networks. *Proc. of the Third Int. ICWSM Conf.* **2009**, *8*, 361−362.

(30) Fruchterman, T.; Reingold, E. Graph drawing by force-directed placement. *Software Pract. Exp.* **1991**, *21*, 1129−1164.

(31) Schloss, P. D.; Westcott, S. L.; Ryabin, T.; Hall, J. R.; Hartmann, M.; Hollister, E. B.; Lesniewski, R. A.; Oakley, B. B.; Parks, D. H.; Robinson, C. J.; Sahl, J. W.; Stres, B.; Thallinger, G. G.; Van Horn, D. J.; Weber, C. F. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **2009**, *75*, 7537−7541.

(32) Huang, S. S.; Labus, B. J.; Samuel, M. C.; Wan, D. T.; Reingold, A. L. Antibiotic resistance patterns of bacterial isolates from blood in San Francisco County, California, 1996−1999. *Emerging Infect. Dis.* **2002**, *8*, 195−201.

(33) Merchant, S.; Tabak, Y. P.; Deryke, C. A.; Depestel, D. D.; Johannes, R. S.; Moise, P.; Gupta, V. *Pseudomonas aeruginosa* non-susceptibility to common antibiotics by source in USA hospitals in 2015: A Multicenter Study. *Open Forum Infectious Diseases*, 2016, *3*, suppl_1.

(34) FDA. *Cephalosporin Order of Prohibition Goes into Effect*; US Department of Health and Human Services, US Food and Drug Administration: Rockville, MD, 2012.

(35) Van Boeckel, T. P.; Gandra, S.; Ashok, A.; Caudron, Q.; Grenfell, B. T.; Levin, S. A.; Laxminarayan, R. Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. *Lancet Infect. Dis.* **2014**, *14*, 742−750.

(36) Hu, H. W.; Han, X. M.; Shi, X. Z.; Wang, J. T.; Han, L. L.; Chen, D.; He, J. Z. Temporal changes of antibiotic-resistance genes and bacterial communities in two contrasting soils treated with cattle manure. *FEMS Microbiol. Eco.* **2016**, *92*, fiv169.

(37) Enright, M. C.; Robinson, D.; Randle, G.; Feil, E. J.; Grundmann, H.; Spratt, B. G. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 7687−7692.

(38) Moran, G. J.; Krishnadasan, A.; Gorwitz, R. J.; Fosheim, G. E.; McDougal, L. K.; Carey, R. B.; Talan, D. A. Methicillin-resistant *S. aureus* infections among patients in the emergency department. *N. Engl. J. Med.* **2006**, *355*, 666−674.

DOI: 10.1021/acs.est.8b02204
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

(39) Chambers, H. F.; Deleo, F. R. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat. Rev. Microbiol.* **2009**, *7*, 629−641.
(40) Garau, J.; Bouza, E.; Chastre, J.; Gudiol, F.; Harbarth, S. Management of methicillin-resistant *Staphylococcus aureus* infections. *Clin. Microbiol. Infect.* **2009**, *15*, 125−136.
(41) Smillie, C. S.; Smith, M. B.; Friedman, J.; Cordero, O. X.; David, L. A.; Alm, E. J. Ecology drives a global network of gene exchange connecting the human microbiome. *Nature* **2011**, *480*, 241−244.
(42) Kan, H.; Zhao, F.; Zhang, X. X.; Ren, H.; Gao, S. Correlations of gut microbial community shift with hepatic damage and growth inhibition of *Carassius auratus* induced by pentachlorophenol exposure. *Environ. Sci. Technol.* **2015**, *49*, 11894−11902.
(43) Newman, M. E. Modularity and community structure in networks. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 8577−8582.
(44) Forsberg, K. J.; Patel, S.; Gibson, M. K.; Lauber, C. L.; Knight, R.; Fierer, N.; Dantas, G. Bacterial phylogeny structures soil resistomes across habitats. *Nature* **2014**, *509*, 612−616.
(45) Sun, S. X.; Wang, H. Use of injections and antibiotics in the outpatients from Xi'an Huashan central hospital during 2008−2010. *Evaluation and Analysis of Drug-Use in Hospitals of China* **2012**, *12*, 889−892.
(46) Pitout, J.; Laupland, K. B. Extended-spectrum β-lactamase-producing *Enterobacteriaceae*: An emerging public-health concern. *Lancet Infect. Dis.* **2008**, *8*, 159−166.
(47) Jácome, P. R.; Alves, L. R.; Jácome-Júnior, A. T.; Silva, M. J.; Lima, J. L.; Araújo, P. S.; Lopes, A. C.; Maciel, M. A. Detection of bla_{SPM-1}, bla_{KPC}, bla_{TEM} and bla_{CTX-M} genes in isolates of *Pseudomonas aeruginosa*, *Acinetobacter* spp. and *Klebsiella* spp. from cancer patients with healthcare associated infections. *J. Med. Microbiol.* **2016**, *65*, 658−665.
(48) Liu, W. J.; Fu, L.; Huang, M.; Zhang, J. P.; Wu, Y.; Zhou, Y. S.; Zeng, J.; Wang, G. X. Frequency of antiseptic resistance genes and reduced susceptibility to biocides in carbapenem-resistant *Acinetobacter baumannii*. *J. Med. Microbiol.* **2016**, *66*, 13−17.
(49) WHO. *Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, And Development of New Antibiotics*; World Health Organization: Geneva, 2017.
(50) Jones, S. R.; Amon, M.; Falvey, C.; Patrick, K. *Serratia marcescens* colonising the gut. *Lancet* **1978**, *1*, 1105.
(51) Sharma, P. K.; Balkwill, D. L.; Frenkel, A.; Vairavamurthy, M. A. A new *Klebsiella planticola* strain (Cd-1) grows anaerobically at high cadmium concentrations and precipitates cadmium sulfide. *Appl. Environ. Microbiol.* **2000**, *66*, 3083−3087.
(52) Sokol, H.; Pigneur, B.; Watterlot, L.; Lakhdari, O.; Bermúdez-Humarán, L. G.; Gratadoux, J. J.; Blugeon, S.; Bridonneau, C.; Furet, J. P.; Corthier, G.; Grangette, C.; Vasquez, N.; Pochart, P.; Trugnan, G.; Thomas, G.; Blottière, H. M.; Doré, J.; Marteau, P.; Seksik, P.; Langella, P. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 16731.
(53) De, G. B.; Vanhaecke, L.; Verstraete, W.; Boon, N. Degradation of acetaminophen by *Delftia tsuruhatensis* and *Pseudomonas aeruginosa* in a membrane bioreactor. *Water Res.* **2011**, *45*, 1829−1837.
(54) Luo, G.; Wang, W.; Angelidaki, I. Anaerobic digestion for simultaneous sewage sludge treatment and CO biomethanation: Process performance and microbial ecology. *Environ. Sci. Technol.* **2013**, *47*, 10685−10693.
(55) Xi'an Municipal Bureau of Statistics and National Bureau of Statistics Survey Office in Xi'an. *Xi'an Statistical Yearbook*; China Statistics Press: Beijing, 2005−2015.
(56) Flanagan, J. L.; Brodie, E. L.; Weng, L.; Lynch, S. V.; Garcia, O.; Brown, R.; Hugenholtz, P.; DeSantix, T. Z.; Andersen, G. L.; Wiener-Kronish, J. P.; Bristow, J. Loss of Bcterial Diversity during Antibiotic Treatment of Intubated Patients Colonized with *Pseudomonas aeruginosa*. *J. Clin. Microbiol.* **2007**, *45*, 1954−1962.
(57) Martínez, J. L. Antibiotics and antibiotic resistance genes in natural environments. *Science* **2008**, *321*, 365−367.
(58) Xiong, W.; Sun, Y.; Ding, X.; Wang, M.; Zeng, Z. Selective pressure of antibiotics on ARGs and bacterial communities in manure-polluted freshwater-sediment microcosms. *Front. Microbiol.* **2015**, *6*, 194.
(59) Hammesfahr, U.; Heur, H.; Manzke, B.; Smalla, K.; Thiele-Bruhn, S. Impact of the antibiotic sulfadiazine and pig manure on the microbial community structure in agricultural soils. *Soil Biol. Biochem.* **2008**, *40*, 1583−1591.
(60) Gutiérrez, I. R.; Watanabe, N.; Harter, T.; Glaser, B.; Radke, M. Effect of sulfonamide antibiotics on microbial diversity and activity in a Californian Mollic Haploxeralf. *J. Soils Sediments* **2010**, *10*, 537−544.
(61) Lu, X. M.; Lu, P. Z. Diversity, abundance, and spatial distribution of riverine microbial communities response to effluents from swine farm versus farmhouse restaurant. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 7597−7608.

DOI: 10.1021/acs.est.8b02204
Environ. Sci. Technol. XXXX, XXX, XXX−XXX