The Abundance and Diversity of Antibiotic Resistance Genes in the Atmospheric Environment of Biology Laboratories and Surroundings

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Research

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Abstract

Background: Antibiotic resistance genes (ARG) have been considered as a global emerging threat to public health systems. Places including farms and hospitals where antibiotics are used, and wastewater treatment plants and landfills where antibiotics are discharged, have been the hot spots for studies. However, locations where ARGs are directly used, such as biology laboratories have been largely neglected.

Methods: In this study, 11 Swiss biology laboratories working on different fields and located in the city center, suburb and rural area were studied to reveal the abundance and diversity of airborne ARGs in them and their surrounding areas with Colony-forming units (CFU) cultivation and quantitative Polymerase Chain Reaction (qPCR).

Results: Most biology laboratories did not discharge significant amounts or varieties of ARGs and cultivate bacteria via air. No correlation was found between the number of CFUs and the abundance of 16S rRNA, but two clusters of correlated airborne ARGs, the animal husbandry related cluster, and city and hospital related cluster were identified in this study.

Conclusions: Although most biology laboratories may not be the emission sources of variety of airborne ARGs, the ARGs in the animal husbandry related cluster which were abundant in the animal laboratories and \textit{aadA1} which was abundant in the laboratories working on other eukaryocyte need to be furtherly studied to make sure if they are potential health risks for the researchers.

Background

In 1928, Alexander Fleming discovered the first antibiotic in the world, the penicillin which saved countless lives and changed animal breeding industries over the last decades. However, 17 years later, in 1945, the same year he won a Nobel prize for this discovery, he warned that abuse of the drug would cause a selection of resistant bacteria in an interview with the New York Times [1]. True to his prediction, within 10 years of the worldwide introduction of penicillin, resistance began to emerge. Nowadays, antibiotic resistance has become a research hotspot and is considered as a global emerging threat to public health systems[2, 3]. Antimicrobial resistance (AMR) has been listed by the United Nations Environment Programme as one of the six global emerging environmental issues[4]. It is estimated that global mortality attributable to AMR will be nearly 700000 per year, and is expected to rise to 10 million annually by 2050[5]. This is not surprising, since the existence of more than 20000 potential antibiotic resistance genes (ARGs) of nearly 400 different types, has been predicted from available bacterial genome sequences[6] and these ARGs have been found in various environments all over the world, including rivers[7-9], lakes[10], coastal areas[7, 11], soil[12, 13], sediments[7, 14], hospitals[15, 16], wastewater treatment plants (WWTPs)[17], aquaculture farms[18-20], livestock farms[21-23], livestock markets[24], composting plants[25], landfills[26-28], and even in the areas with less anthropogenic influences such as the deep ocean[29], Tibet Plateau[30] and permafrost sediments[31]. Among these
environments, animal husbandries, hospitals, WWTPs and landfills are considered as the major known contributors for AMR[9, 22, 26, 32, 33]. These places can be sorted into two types, the former two are the places where antibiotics are used and the latter two are the places where antibiotics and ARGs are discharged. However, there are places where both antibiotics and ARGs are used or even produced, namely pharmaceutical plants, fermentation industry and biological labs. Even though there have been a lot of studies about ARGs and antibiotic resistance bacteria (ARB) detected in pharmaceutical wastewater, the airborne ARGs in these places has rarely been studied. To the authors’ knowledge, only one previous study has researched the existence of ARBs and ARGs in the bioaerosols in pharmaceutical factories [34]. They isolated over 100 strains of ARBs and detected the existence of *blaTEM, blaSHV* and *aphA-1* among these isolates [34]. No quantitative studies on this topic has been done yet.

Antibiotics and ARGs are widely used in these places for cloning, protein production, gene therapy and disease models. Even though autoclaves, UV light and other sterilization methods are commonly used to avoid discharging ARGs from indoor to outdoor environments, ARBs with ARGs were found in the aerosol of clean rooms of drug factories[34]. Therefore, even the clean rooms of labs, pharmaceutical plants and fermentation industry could be potential sources of ARBs and ARGs. What is more, a biology laboratory with lower biological safety level does not have to be a bio-clean room, which means the air is not sterilized. In such laboratories or non-clean rooms of these factories, researchers and workers are likely to be exposed to more ARGs. In fact, one adult in the urban area inhales approximately 0.1-1 μg of DNA per day, which is equivalent to $10^7-10^8$ bacterial cells[35]. Among these DNA copies, the amount of ARGs is significant and similar to the human daily intake from drinking water and accidental ingestion of agricultural soil[36]. Thus, if antibiotic resistant pathogens are present as a part of the inhaled bacteria, they might cause direct damage to human health. A recent study also exhibited that ARG distribution in indoor dust was closely related to the pathogens and antimicrobial drug residuals carried by the dust itself[37]. These evidences call for increased health concerns about airborne ARGs for biological researchers and workers in pharmaceutical plants and fermentation industry who may have more exposure.

Furthermore, airborne transportation of pollutants can also overcome geographical barriers and has been proposed as an important pathway for ARBs and ARGs to disseminate over long distances in the environment[36, 38]. Although because of the low nutrients and water availability in the atmosphere, airborne microbes are usually low in biological activities[39], they can regain their bioactivity and proliferate rapidly in a favorable environment[40, 41]. Therefore, microbes in labs, pharmaceutical plants and fermentation industry are a potential threat to the surroundings.

In addition, ARGs can be acquired not only through self-inheritance, but also through horizontal gene transfer (HGT) from one bacteria to another; or from the environment to human-related commensals and other bacteria with the assistance from mobile genetic elements (MGEs), including plasmids, intergrons, transposes, and phages[42-47]. Airborne microbes from labs, pharmaceutical plants and fermentation industry are more likely to have MGEs than other airborne microbes, since MGEs are commonly used for
cloning and gene editing. This may make these places an even more dangerous ARB and ARG source than other facilities.

Therefore, the present study aims to reveal the abundance and diversity of airborne ARGs in biology laboratories and their surrounding areas. 11 biology laboratories working on different fields and located in the city center, suburb and rural area in Switzerland were studied along with a material laboratory as the control. Colony-forming units (CFU) of airborne bacteria samples after cultivation were counted. Abundances of 22 genes including 17 ARGs and 2 MGEs were measured by qPCR.

Materials And Methods

Sample Collection, Pretreatment and CFU counting

All air samples were collected in independent triplicates from two laboratory buildings and one laboratory building complex and their surroundings which are located in the city center, suburb and rural sites (Figure.1 & Supplementary Table. 1, Additional File.1) in Switzerland with a high flow sampler for assay of airborne microorganisms (dBlueTechR HighBioTrap, Beijing Dingblue Technology, Beijing, China) from September 6th to September 17th, 2019. All laboratory samples were collected inside 11 biology labs, except one was taken inside a material laboratory as control (Figure.1 & Supplementary Table. 1, Additional File.1).

The names of sampling sites in city center start with letter C, the suburbia ones start with S, and the rural ones start with R. For the second letter, L stands for lab, C stands for corridor, O stands for outside. Adjacent corridor samples were taken for each labs, and they have the same number in their names, for example SC2 is the adjacent corridor to SL2. However, two laboratories often shared one corridor in which case the number in the corridor sample name was taken from the laboratory name with the smaller number, such as CL1 and CL2 shared CC1, SL3 and SL4 shared SC31, SL5 and SL6 shared SC5, RL1 and RL3 shared RC11. The numeric “2” in SC32 and RC12 means there was one or two normally closed doors dividing the corridor, and the denoted extra samples were taken on the other side of door opposite to the side of the laboratories. ML stands for material lab. All outdoor samples for each location included at least one site 15m away from the corresponding building or building complex indicated by a name ending with 1 and one site 150m away indicated by a name ending with 2. CO3 at the Polybahn station, a funicular railway station in Zurich was also taken 160m away from the laboratory building to exclude the potential influence of the hospital on CO2. SOB was taken on the balcony connected to the floor of SL1 and SL2 with a normally closed door.

For qPCR, the airborne particle samples were collected on aluminum foils covered with 500 μL mineral oil. The sampler was operated for 30 min at a sampling flow rate of 1000L/min. The aluminum foil was then transferred into a 50 mL falcon tube and centrifuged at 4000 rpm for 2 minutes to collect the mineral oil. 1 mL 0.05% tween-20 water was added to the mineral oil. The mix was incubated for 30 min, then
centrifuged at 7000 rpm for 2 minutes. The aqueous phase was collected into 1.5 mL Eppendorf tubes and stored at -20°C before analysis.

For CFU counting, the airborne particle samples were collected on Lysogeny broth (LB) agar plates. The sampler was operated for 1 min at a sampling flow rate of 1000L/min. The agar plates were cultured at 37°C for 24 hours. The CFUs developed were manually counted, and their averages and standard deviations from three repeats were calculated.

**DNA extraction and real-time quantitative PCR**

DNA was extracted from 250 μL pretreated solution using the PowerSoil DNA Isolation Kits (Mo Bio, Qiagen, Germany) according to the manufacturer's protocol. The quality and quantity of the extracted DNA were determined by gel electrophoresis and an Infinitier 200 PRO plate reader (TECAN, Switzerland).

The occurrence of ARGs and other target genes in the samples was first detected by PCR. 22 pairs of primers of 16 ARGs covering 8 different classes (Supplementary Table. 2, Additional File.1) along with 16S rRNA gene, 2 MGEs (intI1 and TnpA) and 2 Human pathogenic bacteria genes (Staphylococcus spp. and E. coli) were chosen. The PCR reaction mixture was 20 μL, containing 1 μL of DNA template, 14.92 μL of ddH₂O, while the volumes of other components were proportionally according to the protocol of Taq DNA Polymerase, recombinant (Life Technologies, Thermo Fisher, USA). The primers and primer melting temperatures (Tm) are listed in Supplementary Table. 2, Additional File.1. The PCR products were run on 2% agarose gel electrophoresis to detect the presence of the target genes. The products were purified with a QIAquickR gel extraction kit (Qiagen, Germany). The purified genes were cloned into E.coli JM109 with pGEM-T Easy vector system (Promega, USA). Positive clones were randomly selected by blue-white screening method, and then cultivated and checked by PCR. The plasmids were extracted with a Qiaprep spin miniprep kit (Qiagen, Germany) to serve as the standard plasmids for qPCR. The concentration of the extracted plasmids was quantified by Infinitier 200 PRO plate reader (TECAN, Switzerland).

All target genes were quantified by qPCR on a CFX96 Touch™ Real-Time PCR Detection System (BioRad, USA) using SYBR Green I approach. The reaction mixture of qPCR was 10 μL, containing 5 μL SsoAdvanced Universal SYBR Green supermix (BioRad, USA), 0.25 μL of each primers, 0.5 μL of template and 4 μL of ddH₂O. The primers and Tm were the same as the ones for PCR. Purity of the qPCR products was checked using the melting curve method. All measurements were conducted in triplicates. The copy number of each target gene was calculated based on the corresponding standard curve which was set up with tenfold serial dilutions with the above mentioned plasmids carrying corresponding genes.

**Statistical analyses**

The average values, standard deviations of all data and the linear regression of the standard curve were determined with Microsoft Excel 2016. The absolute abundances of functional genes were divided by the absolute abundances of 16S rRNA to get their relative abundances of target genes. All data were added 1 and then taken the logarithm for normalization. All Figures were drawn with Rstudio (v3.6.0, http://www.r-
The heat maps were drawn with the ‘pheatmap’ package\[48\]. The Principal component analysis (PCA) analysis was conducted with the ‘ggplot2’ package\[49\] and ‘ggord’ package\[50\]. The Ternary graphs were drawn based on the average abundance of target genes from the same type of the sampling sites by using the ‘ggplot2’ package\[49\] and ‘ggtern’ package\[51\]. A correlation between two items was considered statistically robust if the Pearson’s correlation coefficient (r) was >0.6 and the P-value was <0.05. The robust pairwise correlations of the target genes formed their co-occurrence networks using the ‘psych’ package\[52\] and ‘igraph’ package\[53\].

**Results And Discussion**

*The variations in the abundance of cultivable bacteria concentration in the air samples of laboratories and surroundings*

Cultivable airborne bacteria concentration of all samples varied from 1 CFU/m\(^3\), sample RL2 to 41 CFU/m\(^3\), sample CO3 (Figure. 2), significantly lower than the cultivable airborne bacteria concentrations of library, hospital, other indoor environmental air samples and outdoor air samples such as wastewater treatment plant measured with tryptic soy agar (TSA) and blood agar (BA) medium in previous studies\[15, 54-56\].

Corridor samples generally had higher CFUs than respective laboratory samples, except SC1. Larger volume of human ow in the corridor may be the main reason, since studies have suggested airborne bacteria emission rate of human breath could be up to 4.85×10\(^5\) CFU/h/person in an air-conditioned room\[54\], and the concentration of airborne bacterial genomes in an occupied classroom was 12-2700 times of that in a vacant room \[57\]. This also applied to CO3, the sampling site with the highest CFU, as the Polybahn station had the largest stream of human ow among all outdoor sampling sites. A potential reason for SC1 to have lower CFUs than SL1 was that SL1 was a very big laboratory with several rooms linked by an internal corridor, thus people used the internal corridor more often than the external corridor.

Cloning experiments were performed in SL2 one day before the sampling, which explained why its cultivable bacteria concentration was the highest among the laboratories and as high as SC2. This suggests laboratories could be the source of airborne bacteria. Even for laboratories like SL6, in which cloning experiments had not been performed for a year, there was still a considerable amount of cultivable airborne bacteria. However, several biology laboratories had less airborne bacteria than the material lab.

*The variation in the abundance of 16S rRNA in the air samples of laboratories and surroundings*

The abundance of 16S rRNA in the air samples of city laboratories and surroundings did not vary too much (Figure. 3 & Supplementary Table. 3 & 4, Additional File.1). CL2 was the lowest one with 7.32×10\(^4\) copies/m\(^3\), while CO1 was the highest with 1.02×10\(^5\) copies/m\(^3\) (Figure. 3 & Supplementary Table. 3 & 4, Additional File.1), which was still lower than most samples from suburbs and all samples from rural area.
The outdoor samples from the suburb were lower in the abundance of 16S rRNA in air than most other suburb samples. The lowest one was SOB, 9.92×10^4 copies/m^3 (Figure. 3 & Supplementary Table. 3 & 4, Additional File.1). Among all the indoor samples, the material laboratory and the physics department corridor SC32 were right in the middle, 1.43×10^5 copies/m^3 and 1.38×10^5 copies/m^3 (Figure. 3 & Supplementary Table. 3 & 4, Additional File.1). Laboratories with frequent cloning experiments such as SL2 and SL3 had the highest abundance of 16S rRNA among all the laboratory samples, and they clearly had an impact on their nearby corridors. Other labs, SL1, SL4, SL5, and SL6 had low abundance of airborne 16S rRNA. For SL5 and SL6, the reason was that they were on the ground floor with open doors to the outside, while SL1 and SL4 had lower concentrations, because more experiments on eukaryocyte instead of bacteria were performed there. This led to decreasing 16S rRNA concentrations from SL2 to SC2, then to SC1 and SL1 which physically comprised one entire floor of the building (Figure. 3 & Supplementary Table. 3 & 4, Additional File.1).

The rural outside samples, RO1 and RO2 had the highest abundance of 16S rRNA among all samples, 2.35×10^5 copies/m^3 and 3.82×10^5 copies/m^3, respectively (Figure. 3 & Supplementary Table. 3 & 4, Additional File.1). Earlier studies suggested in summer and autumn rural areas were heavier in atmospheric bacterial loadings than urban and industrial areas[36]. This made the corridors a valley bottom of atmospheric bacterial loadings. RC12 with lots of open windows was clearly impacted by outside, while RC11 was the lowest, 1.18×10^5 copies/m^3 (Figure. 3 & Supplementary Table. 3 & 4, Additional File.1).

Overall, all the indoor samples were higher in atmospheric bacterial loadings than family residences[58, 59], vacant classrooms, but comparable to occupied classrooms [57, 60]. The outdoor samples were also higher than urban air of Seoul, Colorado, Ji’nan, and Nanjing investigated in several previous studies [36, 59, 61-63], but quite comparable to Beijing, Milan and Berkeley urban air [64-66]. The atmospheric bacterial loading represented by 16S rRNA measured by qPCR showed a totally different pattern from the one exhibited by cultivable airborne bacteria concentration. There was no correlation between them. Different from cultivable airborne bacteria concentration, human flow was not the major contributor for the atmospheric bacteria loading. CO3, the Polybahn station was likely relatively low in uncultivable airborne bacteria and dead bacteria, while rural outside samples were high in these.

The variation in the abundance of ARGs in the air samples of laboratories and surroundings

The only target ARG not detected in any sample was sul1. Other 15 target ARGs were found in almost all the samples, except that there was no floR in RC11 (Figure. 3 & Supplementary Table. 3 & 4, Additional File.1). Based on the absolute concentrations, all target genes can be categorized into 3 groups: the abundant ones with log of copies/m^3 more than 10 in most sites included blaTEM, floR, sul2, aadA1; the rare ones with log of copies/m^3 less than 6 in most sites included aac6II, ermA, qnrS, blaOXA10 and Staphylococcus spp; while the rest target genes fell into the medium group. Clearly, ARGs resistant of the same kind of antibiotics can behave differently. For example, as ARGs against sulfonamide, sul2 was in the abundant group, while sul1 was not detected; as ARGs against aminoglycoside, blaTEM was in the
abundant group, while $blaOXA10$ was in the rare group. The situation was similar for $aadA1$ and $aac(6')II$. In contrast, all target genes against tetracycline and vancomycin were in the medium group. These patterns coincided with the study by Li et al. [67] that $sul1$ could not be detected in Zurich air, but $sul2$ could and $blaTEM$ was the most abundant ARGs. However, ARGs such as $ermA$, $tetW$, which were not detected by Li et al. [67] were found in our study and the relative abundances for ARGs and MGEs showed different characteristics (Figure. 4 & Supplementary Table. 5 & 6, Additional File.1). Compared to Li et al. [67], in our study the relative abundances of $sulII$ and $intI1$ were much higher; $aac(6')II$ and $blaTEM$ varied in a much larger ranges.

Both the absolute and relative abundances of $aac(6')II$ were high in CL2, CC1, SO2, and extremely high in CO2, $2.19\times10^6$ copies/m$^3$ or $26.11$ copies/m$^3$/16S rRNA copies/m$^3$ (Figure. 3 & 4 & Supplementary Table. 3-6, Additional File.1), while in some labs, corridors and all the rural sample sites, its relative abundances were lower than the ones reported in Li et al.[67]. $aac(6')II$ was the only ARG that was extremely high in CO2, which suggested the hospital should be the main source of this specific ARG.

The relative abundance of $blaTEM$ was high in some laboratories and all the suburb and rural outside sites and extremely high in CL2 and CC1, $53.13$ and $40.79$ copies/m$^3$/16S rRNA copies/m$^3$ (Figure. 4 & Supplementary Table. 5 & 6, Additional File.1), respectively, while the relative abundances of $blaTEM$ at all the urban outdoor sites were lower than the level reported for Zurich air by Li et al. [67]. Since their samples were from cabin air filters of cars [67], it is not surprising that their results were between our urban results and suburb results for a city small in area as Zurich. The absolute abundance of $blaTEM$ we detected were quite high compared to the ones in composting plants in Beijing [25], but similar to the ones in other districts including railway stations areas, educational districts, medical districts, residential areas, and commercial districts in Beijing, Tianjin and Shijiazhuang [68].

Other target genes with only high relative abundances in CL2 and CC1 were $floR$, $vanB$, $qnrS$, $qnrA$ and $tetG$ (Figure. 4 & Supplementary Table. 5 & 6, Additional File.1). $qnrS$ and $qnrA$ which are both FCA type ARGs were more abundant both absolutely and relatively in CC1 than CL2 (Figure. 3 & 4 & Supplementary Table. 3-6, Additional File.1). The possible explanation is that some other animal laboratories next to CL2 were the source of these two ARGs. Though the absolute abundances of $qnrS$ in our samples were higher than the ones in Nanjing, China, (Figure. 3 & Supplementary Table. 3 & 4, Additional File.1), the relative ones in our samples, apart from those in CL2 and CC1, were actually lower (Figure. 4 & Supplementary Table. 5 & 6, Additional File.1) [36], and fell into the range of the ones in the Eastern Mediterranean [69]. For $tetG$, all abundances in our samples were higher than the ones in composting plants in Beijing [25], hospitals and farms in Ningbo[16]. Most ones were higher than the ones in districts including railway stations areas, educational districts, medical districts, residential areas, and commercial districts in cities in Northern China[68], but they were comparable to the ones in Chinese wet markets in Shenzhen[24]. The air from live poultry market had about $7.50 \log(copies/m^3)$ $tetG$ [24], which was even higher than the values in CL2 and CC1, $3.05\times10^6$ and $3.65\times10^6$ copies/m$^3$, respectively (Figure. 3 & Supplementary Table. 3 & 4, Additional File.1). The results suggested that animal laboratories like CL2, could be a source of these ARGs because of their frequent contact with experimental animals in their research.
Both tetW and sulII were widely detected in previous studies. The absolute abundances of tetW in our samples were higher than the ones in districts including railway stations areas, educational districts, medical districts, residential areas, and commercial districts [36, 68], composting plants [25], clinics [62], and concentrated swine feeding operation [62], but comparable to concentrated poultry feeding operations [70]. Its relative abundances in our samples were between 0.027 to 0.27 copies/m³/16S rRNA copies/m³ (Figure. 4 & Supplementary Table. 5 & 6, Additional File.1), similar to the ones in Nanjing[36]. The relative abundances of sulII in our samples were higher than the ones in Zurich air reported in Li et al. [67]. The absolute ones were higher than the ones in composting plants, and comparable to the ones in districts including railway stations areas, educational districts, medical districts, residential areas, and commercial districts in Beijing, Tianjin, Shijiazhuang [68] and in Chinese wet markets in Shenzhen[24].

Other ARGs, such as ermA and acrA were also more abundant in our samples compared to the ones in hospitals and farms in Ningbo [16], even though both of them did not belong in the abundant group in our analysis (Figure. 3 & Supplementary Table. 3 & 4, Additional File.1).

The variation in the abundance of MGEs and human pathogen bacteria (HPBs) in the air samples of laboratories and surroundings

Both MGEs were in the abundant target gene group (Figure. 3 & Supplementary Table. 3 & 4, Additional File.1), but the relative abundance of TnpA was much higher than 1.00 copies/m³/16S rRNA copies/m³ at many sites, while intl1 was higher than 1.00 copies/m³/16S rRNA copies/m³ only in CL2, CC1, ML, SL3, RL1 and RC11 (Figure. 4 & Supplementary Table. 5 & 6, Additional File.1). Nevertheless the intl1's relative and absolute abundances in our samples were higher than those in previous studies [25, 62, 67, 69]. Especially, in the study of Li et al. [67], TnpA was not detected on air cabinet filters of automobiles in 14 cities including Zurich among all 19 cities distributed over the world. This contradiction could be caused by the property of TnpA that it can be more easily degraded than other target genes based on our experience. Our samples were freshly collected whereas the samples in Li et al. [67] were accumulated on cabin air filters on cars. Among our samples, TnpA was the highest in CO3 and CO1. It was also higher in SO1 and RO1 than in SO2 and RO2 (Figure. 4 & Supplementary Table. 5 & 6, Additional File.1). These results lead to a hypothesis that TnpA may be contributed by large volume of human flow.

Staphylococcus. Spp belonged to the rare group in our study with the abundances varying from 41.85 to 521.99 copies/m³, while E.coli was in the medium group with the abundances varying from 3580.56 to 26893.71 copies/m³ (Figure. 3 & Supplementary Table. 3 & 4, Additional File.1). The range of Staphylococcus. Spp in our samples was similar to its range in composting plants[25], while E.coli's abundance was close to concentrated poultry feeding operations[70], and higher than the composting plants[25].

The PCA analysis of target genes in the air samples of laboratories and surroundings
In PCA analysis (Figure. 5), Axis2 could be largely explained by the location factor from city to rural area, while Axis1 could be partially explained by the environment factor if CC1 and CL2 were excluded: biology laboratories and city outdoor sites were slightly on the right side with the suburb and rural outdoor sites slightly on the left. ermA, mphA2, aac(6)’-II and Staphylococcus. Spp had more contribution from the city samples. The first three were exclusively contributed by the hospital, since they were only extremely relatively abundant in CO2, the site near the hospital (Figure. 4 & Supplementary Table. 5 & 6, Additional File.1) while tetW, sulII, 16S rRNA and E.coli had more contribution from suburb and rural outdoor samples. CC1 and CL2 mainly contributed qnrS, qnrA, blaOXA10, tetG and intI1, while aadA1 and TnpA pointed to the opposite direction. The results show that animal laboratories were abundant in ARGs related to animal use as expected, while other laboratories were more likely to be the source of aadA1. aadA1 encodes protein which can inactivate aminoglycoside antibiotics. Due to the fact that aminoglycoside antibiotics can cause toxic side effects to inner ear and are contraindicated in patients with myasthenia gravis and mitochondrial disease, they are reluctantly used for medical purposes, but streptomycin, kanamycin are very commonly used in biology laboratories for experiments such as cloning. Furthermore, during the sampling, we were also informed that neomycin and ribostamycin were used in laboratory SL1 where immunology was studied. Since SL4 is the cell room for a structural biology laboratory and CL1 mainly studies fungi, a hypothesis is that biology laboratories working on eukaryocyte may release aadA1.

The co-occurrence network of ARGs, MGEs and HPBs independent of sites

Though previous studies did find the correlations between 16S rRNA and certain ARGs or MGEs in air, water, sediment and soil samples [8, 23, 36], there were no correlations found in this study between 16S rRNA and other target genes analyzed by Pearson Correlation Coefficient.

The correlations between other target genes based on their relative abundances clustered into two groups (Figure. 6): the city and hospital related group consisting of aac(6)’-II, ermA and mphA2, the rural and animal husbandry related group consisting of 8 ARGs, E.coli and intI1. Notably, aadA1 was the only ARG with negative correlations with other five ARGs which happened to point to the counter direction as aadA1 did in the PCA analysis. The other 7 ARGs in the group were strongly positively correlated with each other. The correlations between MGEs, intI1 and ARGs including qnrS, qnrA and blaOXA10 suggest these ARGs may have a higher risk to transfer horizontally. These correlations are consistent with previous studies[20]. However, our study did not find TnpA and sulII correlate with other target genes like previous studies did in different environments[7, 23, 67], this suggests that more ARGs and the co-occurrence between ARGs and antibiotic residuals in aerosol of biology labs, pharmaceutical plants, fermentation industry should be taken into account in future studies.

Conclusion

Though frequent cloning experiments may potentially increase the abundance of cultivable bacteria in air, biology laboratories air did not always contain more cultivable bacteria than the corridor or outside air.
Instead, heavy human flows may be the main source of cultivable airborne bacteria, while more uncultivable airborne bacteria were found in rural outdoors.

No correlation was found between the number of CFUs and the abundance of 16S rRNA, but two clusters of correlated airborne ARGs, the animal husbandry related cluster, and city and hospital related cluster were identified in this study.

Generally speaking, biology laboratories we investigated do not discharge significant amounts and varieties of airborne ARGs. However, most ARGs positively correlated with each other in the animal husbandry related cluster were abundant in animal laboratory and nearby corridor air, while the negatively correlated \(\text{aadA1}\) was richer in biology laboratories working on eukaryocyte. It remains to be established if this would pose a potential health risk for researchers in these bioresearch. More ARGs, HPB and environmental pollutants such airborne antibiotic residuals can be taken into account with other techniques like sequencing, flow cytometry and fluorescence \textit{in Situ} hybridization in future studies to investigate the potential risks for researchers and workers in biology labs, pharmaceutical plants and fermentation factories in more depth.


declarations

Authors’ contributions

Y.Y instructed the sampling. Y.T performed the sampling, CFU counting, qPCR and statistical analyses. J.W supervised the study. Y.T, Y.Y and J.W wrote the manuscript. The authors read and approve the final manuscript.

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Availability of data and materials

The dataset supporting the conclusions of this article are included within the article and its additional file.

Ethics approval and consent to participate

Not applicable.
Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. The Landscape of Antibiotic Resistance. *Environ Health Persp* 2009, **117**(6):A244-A247.

2. Arias CA, Murray BE: Antibiotic-Resistant Bugs in the 21st Century – A Clinical Super-Challenge. *New Engl J Med* 2009, **360**(5):439-443.

3. Ferri M, Ranucci E, Romagnoli P, Giacone V: Antimicrobial resistance: A global emerging threat to public health systems. *Crit Rev Food Sci* 2017, **57**(13):2857-2876.

4. Frontiers 2017: Emerging issues of environmental concern [https://wedocs.unep.org/handle/20.500.11822/22255]

5. Resistance RoA: Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations. In: The Wellcome Trust; 2014.

6. Liu B, Pop M: ARDB-Antibiotic Resistance Genes Database. *Nucleic Acids Res* 2009, **37**:D443-D447.

7. Li A, Chen L, Zhang Y, Tao Y, Xie H, Li S, Sun W, Pan J, He Z, Mai C et al. Occurrence and distribution of antibiotic resistance genes in the sediments of drinking water sources, urban rivers, and coastal areas in Zhuhai, China. *Environ Sci Pollut R* 2018, **25**(26):26209-26217.

8. Luo Y, Mao DQ, Rysz M, Zhou DX, Zhang HJ, Xu L, Alvarez PJJ: Trends in Antibiotic Resistance Genes Occurrence in the Haihe River, China. *Environ Sci Technol* 2010, **44**(19):7220-7225.

9. Pruden A, Pei R, Storteboom H, Carlson KH: Antibiotic Resistance Genes as Emerging Contaminants: Studies in Northern Colorado†. *Environ Sci Technol* 2006, **40**(23):7445-7450.

10. Bengtsson-Palme J, Boulund F, Fick J, Kristiansson E, Larsson DGJ: Shotgun metagenomics reveals a wide array of antibiotic resistance genes and mobile elements in a polluted lake in India. *Front Microbiol* 2014, **5**.

11. Di Cesare A, Vignaroli C, Luna GM, Pasquaroli S, Biavasco F: Antibiotic-Resistant Enterococci in Seawater and Sediments from a Coastal Fish Farm. *Microb Drug Resist* 2012, **18**(5):502-509.

12. Zhang YJ: Prevalence and Transmission of Antibiotic Resistance Genes in the Soil Environment. Melbourne: The University of Melbourne; 2019.

13. Knapp CW, Dolfing J, Ehler PAl, Graham DW: Evidence of Increasing Antibiotic Resistance Gene Abundances in Archived Soils since 1940. *Environ Sci Technol* 2010, **44**(2):580-587.

14. Himmelsbach M, Buchberger W: Residue analysis of oxytetracycline in water and sediment samples by high-performance liquid chromatography and immunochemical techniques. *Microchim Acta* 2005, **151**(1-2):67-72.
15. Gilbert Y, Veillette M, Duchaine C: Airborne bacteria and antibiotic resistance genes in hospital rooms. *Aerobiologia* 2010, **26**(3):185-194.

16. Li Y, Liao H, Yao H: Prevalence of Antibiotic Resistance Genes in Air-Conditioning Systems in Hospitals, Farms, and Residences. *Int J Environ Res Public Health* 2019, **16**(5):683.

17. Mao DQ, Yu S, Rysz M, Luo Y, Yang FX, Li FX, Hou J, Mu QH, Alvarez PJJ: Prevalence and proliferation of antibiotic resistance genes in two municipal wastewater treatment plants. *Water Res* 2015, **85**:458-466.

18. Moore JE, Huang JH, Yu PB, Ma CF, Moore PJA, Millar BC, Goldsmith CE, Xu JR: High diversity of bacterial pathogens and antibiotic resistance in salmonid fish farm pond water as determined by molecular identification employing 16S rDNA PCR, gene sequencing and total antibiotic susceptibility techniques. *Ecotox Environ Safe* 2014, **108**:281-286.

19. Xiong W, Sun Y, Zhang T, Ding X, Li Y, Wang M, Zeng Z: Antibiotics, Antibiotic Resistance Genes, and Bacterial Community Composition in Fresh Water Aquaculture Environment in China. *Microb Ecol* 2015, **70**(2):425-432.

20. Wang JH, Lu J, Wu J, Zhang Y, Zhang C: Proliferation of antibiotic resistance genes in coastal recirculating mariculture system. *Environ Pollut* 2019, **248**:462-470.

21. Wu B, Qi Q, Zhang X, Cai Y, Yu G, Lv J, Gao L, Wei L, Chai T: Dissemination of *Escherichia coli* carrying plasmid-mediated quinolone resistance (PMQR) genes from swine farms to surroundings. *Sci Total Environ* 2019, **665**:33-40.

22. Zhu YG, Johnson TA, Su JQ, Qiao M, Guo GX, Stedtfeld RD, Hashsham SA, Tiedje JM: Diverse and abundant antibiotic resistance genes in Chinese swine farms. *P Natl Acad Sci USA* 2013, **110**(9):3435-3440.

23. Sun M, Ye M, Wu J, Feng Y, Wan J, Tian D, Shen F, Liu K, Hu F, Li H *et al.*: Positive relationship detected between soil bioaccessible organic pollutants and antibiotic resistance genes at dairy farms in Nanjing, Eastern China. *Environ Pollut* 2015, **206**:421-428.

24. Gao X-L, Shao M-F, Luo Y, Dong Y-F, Ouyang F, Dong W-Y, Li J: Airborne bacterial contaminations in typical Chinese wet market with live poultry trade. *Sci Total Environ* 2016, **572**:681-687.

25. Gao M, Qiu T, Sun Y, Wang X: The abundance and diversity of antibiotic resistance genes in the atmospheric environment of composting plants. *Environ Int* 2018, **116**:229-238.

26. Wang PL, Wu D, You XX, Li WY, Xie B: Distribution of antibiotics, metals and antibiotic resistance genes during landfilling process in major municipal solid waste landfills. *Environ Pollut* 2019, **255**.

27. Wu D, Huang XH, Sun JZ, Graham DW, Xie B: Antibiotic Resistance Genes and Associated Microbial Community Conditions in Aging Landfill Systems. *Environ Sci Technol* 2017, **51**(21):12859-12867.

28. Zhang XH, Xu YB, He XL, Huang L, Ling JY, Zheng L, Du QP: Occurrence of antibiotic resistance genes in landfill leachate treatment plant and its effluent-receiving soil and surface water. *Environ Pollut* 2016, **218**:1255-1261.

29. Chen BW, Yang Y, Liang XM, Yu K, Zhang T, Li XD: Metagenomic Profiles of Antibiotic Resistance Genes (ARGs) between Human Impacted Estuary and Deep Ocean Sediments. *Environ Sci Technol*
2013, 47(22):12753-12760.

30. Chen BW, Yuan K, Chen X, Yang Y, Zhang T, Wang YW, Luan TG, Zou SC, Li XD: Metagenomic Analysis Revealing Antibiotic Resistance Genes (ARGs) and Their Genetic Compartments in the Tibetan Environment. Environ Sci Technol 2016, 50(13):6670-6679.

31. D’Costa VM, King CE, Kalan L, Morar M, Sung WWL, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R et al: Antibiotic resistance is ancient. Nature 2011, 477(7365):457-461.

32. Pal C, Bengtsson-Palme J, Kristiansson E, Larsson DGJ: The structure and diversity of human, animal and environmental resistomes. Microbiome 2016, 4.

33. Li C, Lu JJ, Liu J, Zhang GL, Tong YB, Ma N: Exploring the correlations between antibiotics and antibiotic resistance genes in the wastewater treatment plants of hospitals in Xinjiang, China. Environ Sci Pollut R 2016, 23(15):15111-15121.

34. Hamdy AM, El-Massry M, Kashef MT, Amin MA, Aziz RK: Toward the Drug Factory Microbiome: Microbial Community Variations in Antibiotic-Producing Clean Rooms. OMICS: J Integrative Biol 2018, 22(2):133-144.

35. Frohlich-Nowoisky J, Kampf CJ, Weber B, Huffman JA, Pohlker C, Andrae MO, Lang-Yona N, Burrows SM, Gunthe SS, Elbert W et al: Bioaerosols in the Earth system: Climate, health, and ecosystem interactions. Atmos Res 2016, 182:346-376.

36. Xie J, Jin L, Luo X, Zhao Z, Li X: Seasonal Disparities in Airborne Bacteria and Associated Antibiotic Resistance Genes in PM2.5 between Urban and Rural Sites. Environ Sci Tech Let 2018, 5(2):74-79.

37. Hartmann EM, Hickey R, Hsu T, Roman CMB, Chen J, Schwager R, Kline J, Brown GZ, Halden RU, Huttenhower C et al: Antimicrobial Chemicals Are Associated with Elevated Antibiotic Resistance Genes in the Indoor Dust Microbiome. Environ Sci Technol 2016, 50(18):9807-9815.

38. McEachran AD, Blackwell BR, Hanson JD, Wooten KJ, Mayer GD, Cox SB, Smith PN: Antibiotics, Bacteria, and Antibiotic Resistance Genes: Aerial Transport from Cattle Feed Yards via Particulate Matter. Environ Health Persp 2015, 123(4):337-343.

39. Wei K, Zou ZL, Yao MS: Charge levels and Gram (+/-) fractions of environmental bacterial aerosols. J Aerosol Sci 2014, 74:52-62.

40. Albrecht A, Witzenberger R, Bernzen U, Jackel U: Detection of airborne microbes in a composting facility by cultivation based and cultivation-independent methods. Ann Agr Env Med 2007, 14(1):81-85.

41. Womack AM, Bohannan BJM, Green JL: Biodiversity and biogeography of the atmosphere. Philos T R Soc B 2010, 365(1558):3645-3653.

42. Gilchrist MJ, Greko C, Wallinga DB, Beran GW, Riley DG, Thorne PS: The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. Environ Health Persp 2007, 115(2):313-316.

43. Balcazar JL: Bacteriophages as Vehicles for Antibiotic Resistance Genes in the Environment. Plos Pathog 2014, 10(7).
44. Bennett PM: **Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria.** *Brit J Pharmacol* 2008, **153**:S347-S357.

45. Brown-Jaque M, Calero-Caceres W, Muniesa M: **Transfer of antibiotic-resistance genes via phage-related mobile elements.** *Plasmid* 2015, **79**:1-7.

46. Chen B, Liang X, Nie X, Huang X, Zou S, Li X: **The role of class I integrons in the dissemination of sulfonamide resistance genes in the Pearl River and Pearl River Estuary, South China.** *J Hazard Mater* 2015, **282**:61-67.

47. Norman A, Hansen LH, Sorensen SJ: **Conjugative plasmids: vessels of the communal gene pool.** *Philos T R Soc B* 2009, **364**(1527):2275-2289.

48. Kolde R: **pheatmap: Pretty Heatmaps.** In. https://CRAN.R-project.org/package=pheatmap: R Package Version 1.0.12; 2010.

49. Wickham H: **ggplot2.** *Wires* 2011, **3**:180-185.

50. Beck MW: **ggord: Ordination Plots with ggplot2.** R package version 1.0.0. In. https://zenodo.org/badge/latestdoi/35334615; 2017.

51. Hamilton NE, Ferry M: **ggtern: Ternary Diagrams Using ggplot2.** *J Stat Softw* 2018, **87**(Code Snippet 3).

52. Revelle W: **psych: Procedures for Psychological, Psychometric, and Personality Research.** In. https://CRAN.R-project.org/package=psych: R Package Version 1.8; 2018.

53. Gabor Csardi TN: **The igraph software package for complex network research.** *Int J Complex Syst* 2006, **1695**.

54. Priyamvada H, Priyanka C, Singh RK, Akila M, Ravikrishna R, Gunthe SS: **Assessment of PM and bioaerosols at diverse indoor environments in a southern tropical Indian region.** *Build Environ* 2018, **137**:215-225.

55. Goh I, Obbard JP, Viswanathan S, Huang Y: **Airborne bacteria and fungal spores in the indoor environment - A case study in Singapore.** *Acta Biotechnol* 2000, **20**(1):67-73.

56. Li J, Zhou L, Zhang X, Xu C, Dong L, Yao M: **Bioaerosol emissions and detection of airborne antibiotic resistance genes from a wastewater treatment plant.** *Atmos Environ* 2016, **124**:404-412.

57. Qian J, Hospodsky D, Yamamoto N, Nazaroff WW, Peccia J: **Size-resolved emission rates of airborne bacteria and fungi in an occupied classroom.** *Indoor air* 2012, **22**(4):339-351.

58. Emerson JB, Keady PB, Clements N, Morgan EE, Awerbuch J, Miller SL, Fierer N: **High temporal variability in airborne bacterial diversity and abundance inside single-family residences.** *Indoor air* 2017, **27**(3):576-586.

59. Clements N, Keady P, Emerson J, Fierer N, Miller S: **Seasonal Variability of Airborne Particulate Matter and Bacterial Concentrations in Colorado Homes.** *Atmosphere* 2018, **9**(4):133.

60. Hospodsky D, Qian J, Nazaroff WW, Yamamoto N, Bibby K, Rismani-Yazdi H, Peccia J: **Human occupancy as a source of indoor airborne bacteria.** *PLoS One* 2012, **7**(4):e34867.
61. Lee SH, Lee HJ, Kim SJ, Lee HM, Kang H, Kim YP: Identification of airborne bacterial and fungal community structures in an urban area by T-RFLP analysis and quantitative real-time PCR. *Sci Total Environ* 2010, **408**(6):1349-1357.

62. Ling AL, Pace NR, Hernandez MT, LaPara TM: Tetracycline resistance and Class 1 integron genes associated with indoor and outdoor aerosols. *Environ Sci Tech Let* 2013, **47**(9):4046-4052.

63. Xu C, Wei M, Chen J, Wang X, Zhu C, Li J, Zheng L, Sui G, Li W, Wang W et al: Bacterial characterization in ambient submicron particles during severe haze episodes at Ji'nan, China. *Sci Total Environ* 2017, **580**:188-196.

64. Lymperopoulou DS, Adams RI, Lindow SE: Contribution of Vegetation to the Microbial Composition of Nearby Outdoor Air. *Appl Environ Microbiol* 2016, **82**(13):3822-3833.

65. Bertolini V, Gandolfi I, Ambrosini R, Bestetti G, Innocente E, Rampazzo G, Franzetti A: Temporal variability and effect of environmental variables on airborne bacterial communities in an urban area of Northern Italy. *Appl Microbiol Biotechnol* 2013, **97**(14):6561-6570.

66. Zhen Q, Deng Y, Wang Y, Wang X, Zhang H, Sun X, Ouyang Z: Meteorological factors had more impact on airborne bacterial communities than air pollutants. *Sci Total Environ* 2017, **601-602**:703-712.

67. Li J, Cao J, Zhu Y-g, Chen Q-I, Shen F, Wu Y, Xu S, Fan H, Da G, Huang R-j et al: Global Survey of Antibiotic Resistance Genes in Air. *Environ Sci Technol* 2018, **52**(19):10975-10984.

68. Zhou H, Wang X, Li Z, Kuang Y, Mao D, Luo Y: Occurrence and Distribution of Urban Dust-Associated Bacterial Antibiotic Resistance in Northern China. *Environ Sci Tech Let* 2018, **5**(2):50-55.

69. Mazar Y, Cytryn E, Erel Y, Rudich Y: Effect of Dust Storms on the Atmospheric Microbiome in the Eastern Mediterranean. *Environ Sci Tech Let* 2016, **50**(8):4194-4202.

70. 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.

**Figures**
Figure 1

Location of sampling sites (Red: biology lab; yellow: corridor; green: material lab; light blue: outside within 15m from the building; dark blue: outside 150m away from the building)
Figure 2

The CFU numbers of all air samples
Figure 3

The absolute abundance of target genes in all air samples (log(copies/m3+1))
Figure 4

The relative abundance of target genes in all air samples
Figure 5

PCA analysis for the relative abundances of all target airborne genes
Figure 6

Co-occurrence network of ARGs, MGEs and HPBs based on Pearson Correlation coefficient independent of sampling sites (orange lines stand for positive correlation, blue lines stand for negative correlation, the width of the lines stands for the size of the coefficient.)

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