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 *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 Infiniter 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 ddH_2O, 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 Infiniter 200 PRO plate reader (TECAN, Switzerland).

All target genes were quantified by qPCR on a CFX96 Touch^{TM} 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 ddH_2O. 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-

project.org/). 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 flow 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 flow 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, 2.19×10^6 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, 3.05×10^6 and 3.65×10^6 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/m^3/16S rRNA copies/m^3 (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/m^3/16S rRNA copies/m^3 at many sites, while *intI1* was higher than 1.00 copies/m^3/16S rRNA copies/m^3 only in CL2, CC1, ML, SL3, RL1 and RC11 (Figure. 4 & Supplementary Table. 5 & 6, Additional File.1). Nevertheless the *intI1*'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/m^3, while *E.coli* was in the medium group with the abundances varying from 3580.56 to 26893.71 copies/m^3 (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')-Il* 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')-Il, 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 *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 *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.

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Figures

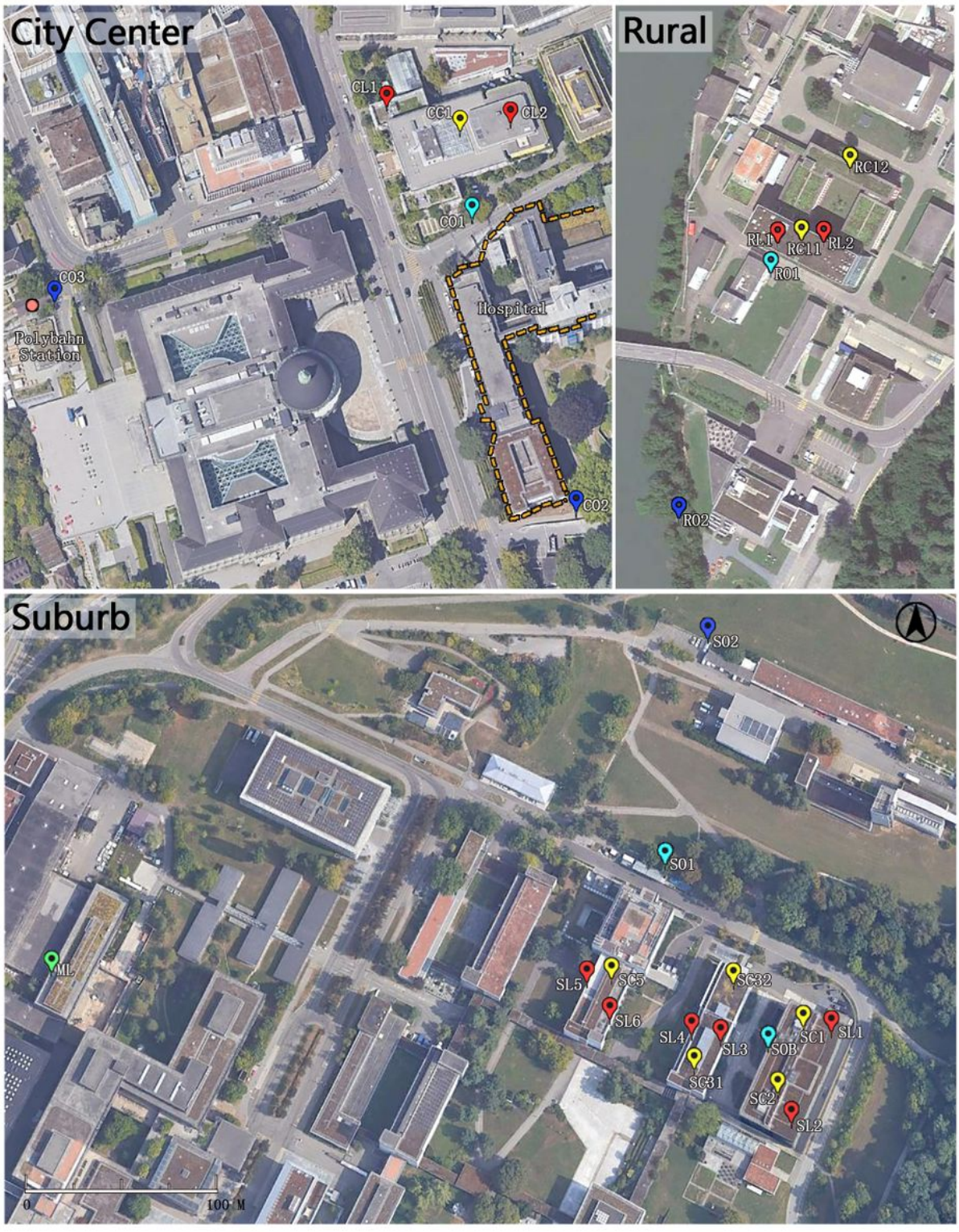

Figure 1

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)

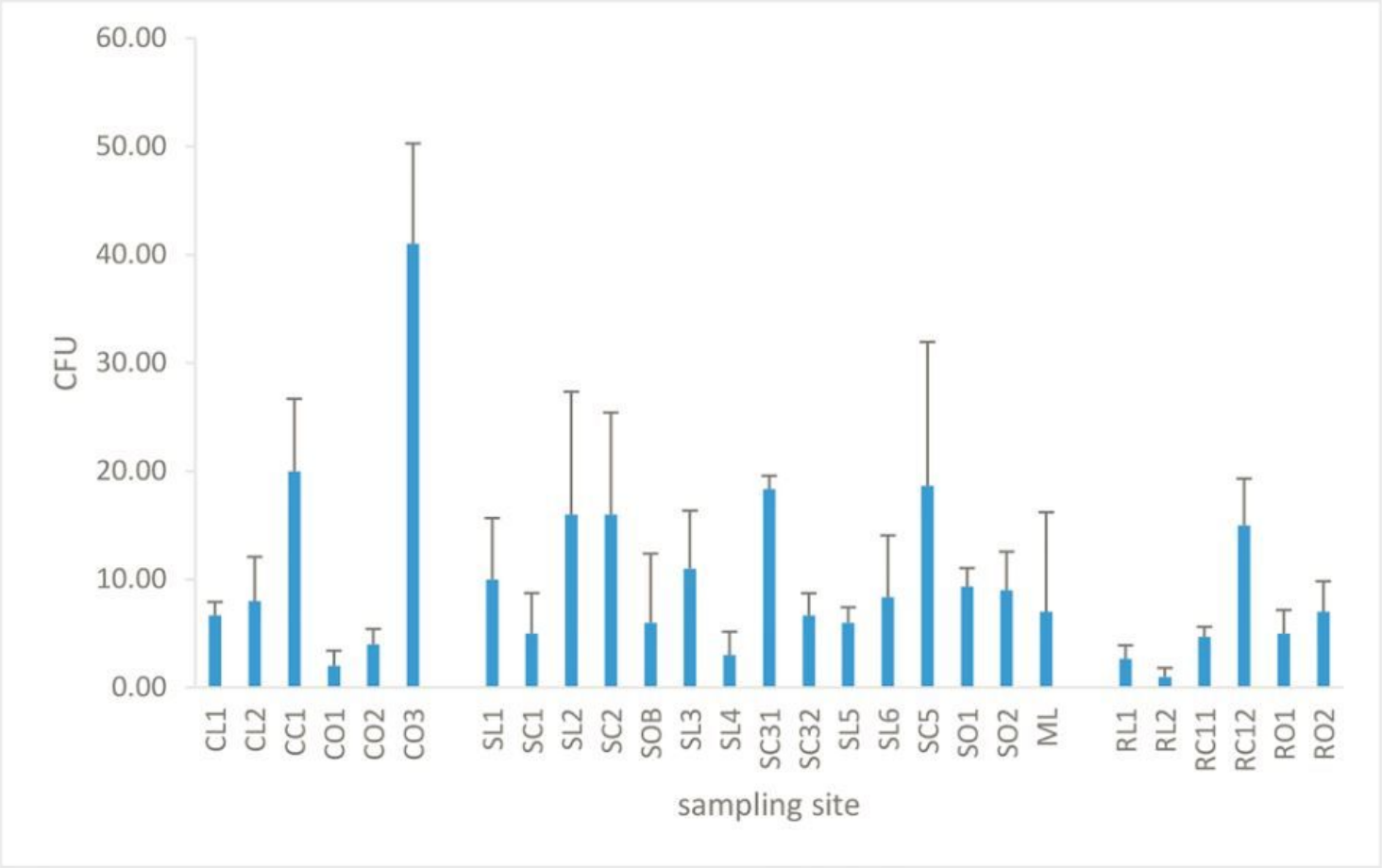

Figure 2

The CFU numbers of all air samples

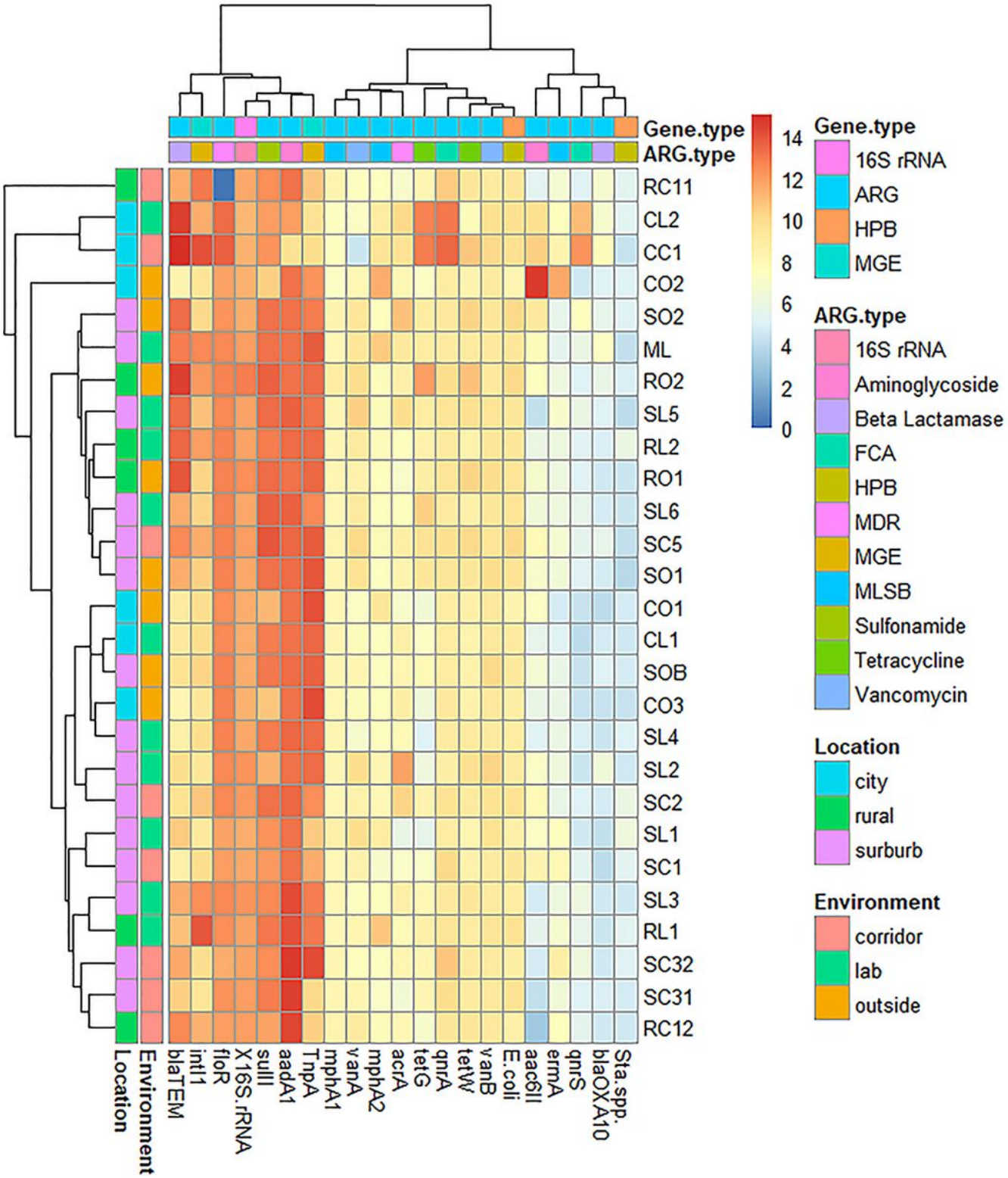

Figure 3

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

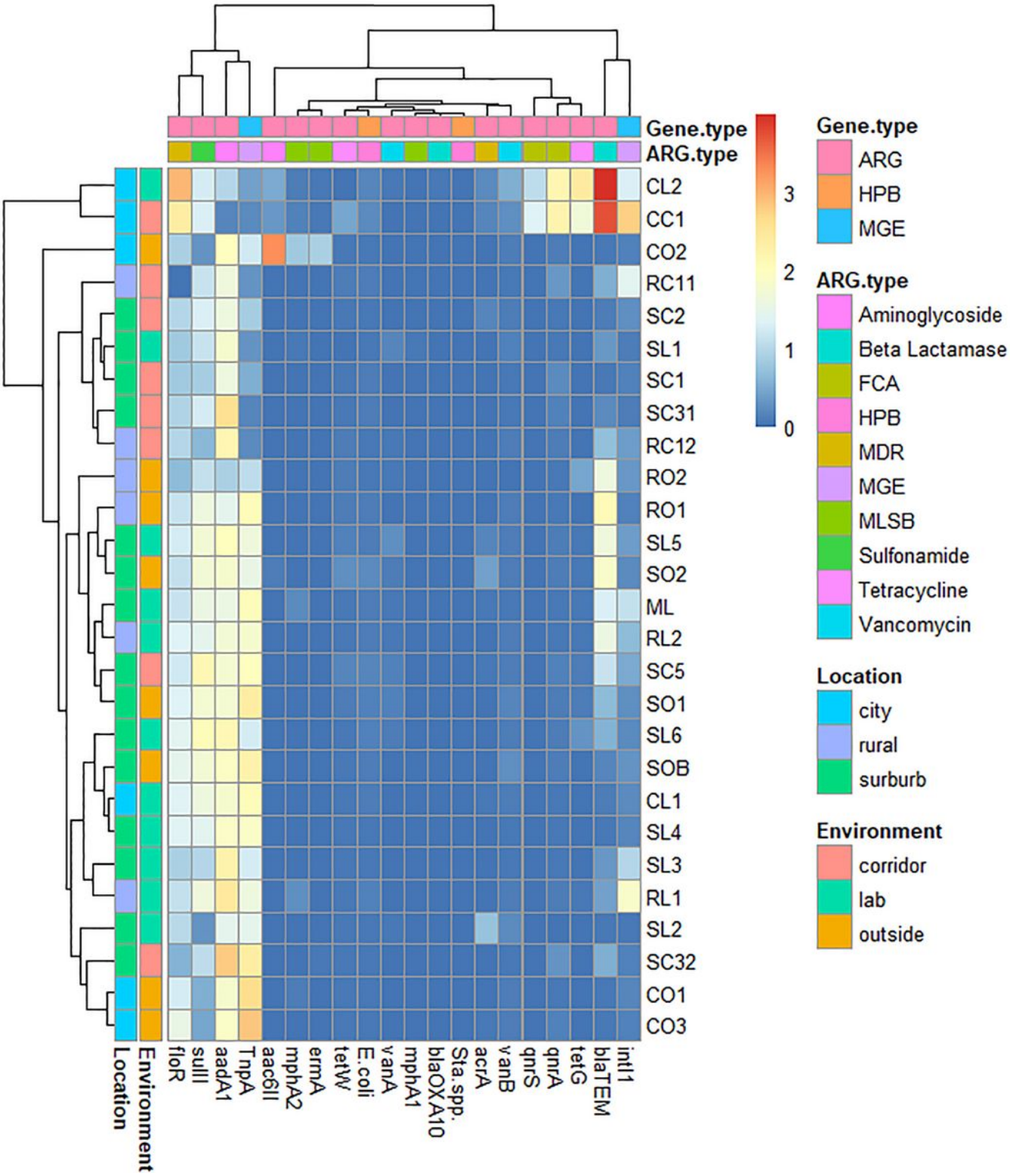

Figure 4

The relative abundance of target genes in all air samples

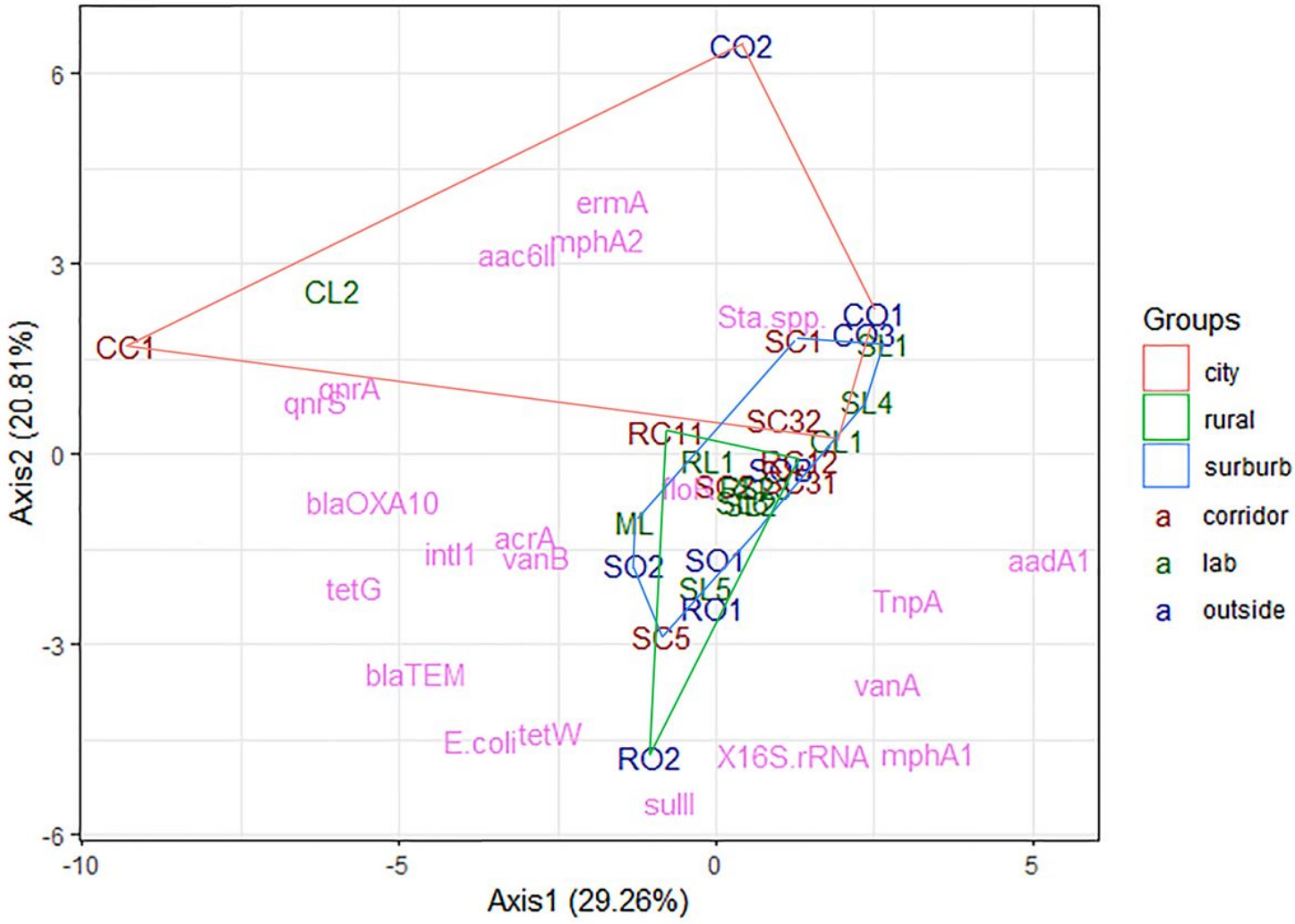

Figure 5

PCA analysis for the relative abundances of all target airborne genes

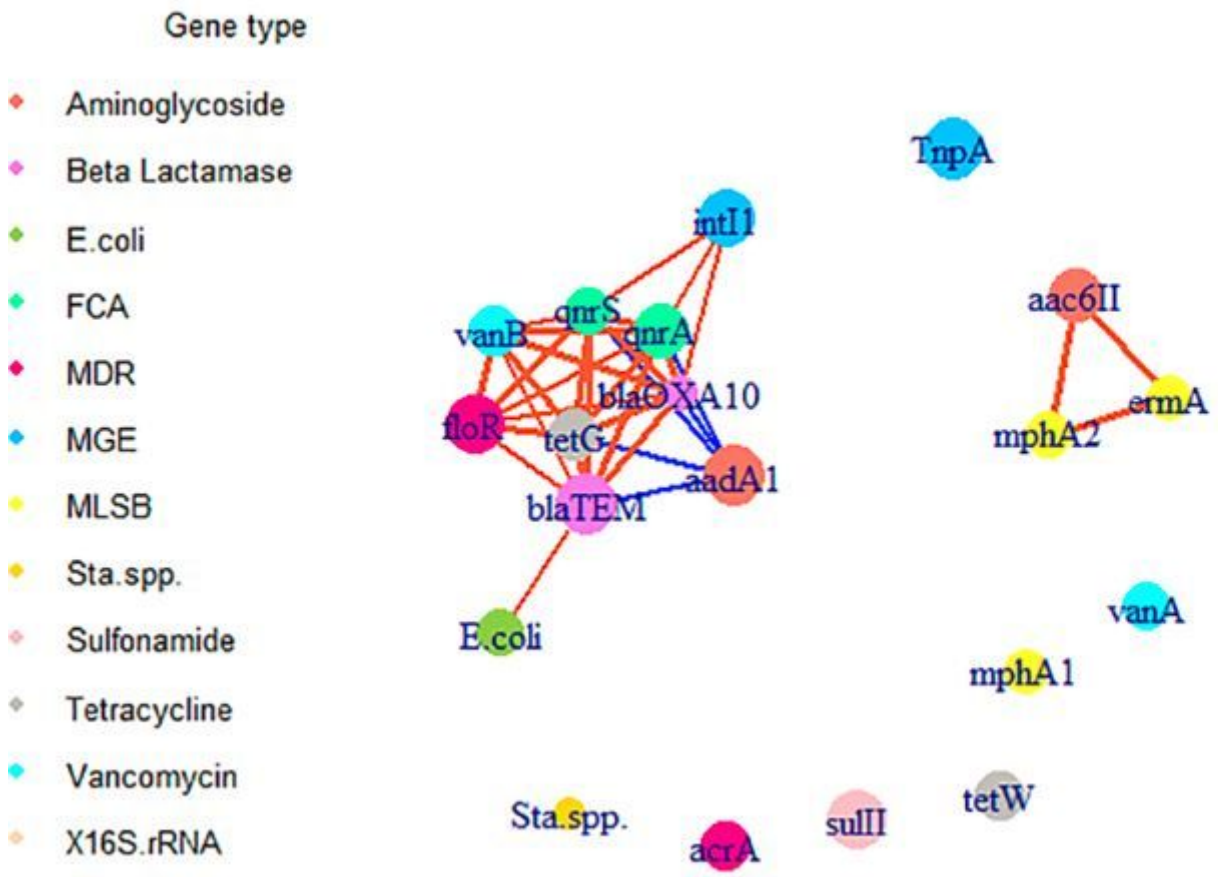

Figure 6

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.)

Supplementary Files

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