



Transmission of human-pet antibiotic resistance via aerosols in pet hospitals of Changchun

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ABSTRACT

In recent years, aerosols have been recognized as a prominent medium for the transmission of antibiotic-resistant bacteria and genes. Among these, particles with a particle size of 2 μm (PM_{2.5}) can directly penetrate the alveoli. However, the presence of antibiotic-resistant genes in aerosols from pet hospitals and the potential risks posed by antibiotic-resistant bacteria in these aerosols to humans and animals need to be investigated. In this study, cefotaxime-resistant bacteria were collected from 5 representative pet hospitals in Changchun using a Six-Stage Andersen Cascade Impactor. The distribution of bacteria in each stage was analyzed, and bacteria from stage 5 and 6 were isolated and identified. Minimal inhibitory concentrations of isolates against 12 antimicrobials were determined using broth microdilution method. Quantitative Polymerase Chain Reaction was employed to detect resistance genes and mobile genetic elements that could facilitate resistance spread. The results indicated that ARBs were enriched in stage 5 (1.1–2.1 μm) and stage 3 (3.3–4.7 μm) of the sampler. A total of 159 isolates were collected from stage 5 and 6. Among these isolates, the genera *Enterococcus* spp. (51%), *Staphylococcus* spp. (19%), and *Bacillus* spp. (14%) were the most prevalent. The isolates exhibited the highest resistance to tetracycline and the lowest resistance to cefquinome. Furthermore, 56 (73%) isolates were multidrug-resistant. Quantitative PCR revealed the expression of 165 genes in these isolates, with mobile genetic elements showing the highest expression levels. In conclusion, PM_{2.5} from pet hospitals harbor a significant number of antibiotic-resistant bacteria and carry mobile genetic elements, posing a potential risk for alveolar infections and the dissemination of antibiotic resistance genes.

1. Introduction

Microbial aerosols are dispersed systems formed by the combination of particulate matter in the air with bacteria, viruses, fungi, and endotoxins [1]. The primary sources of microbial aerosols include microorganisms released from humans and animals through coughing, sneezing, and desquamation [2]. Exposure to microbial aerosols has been associated with air pollution, leading to allergies, cancers, respiratory, and

infectious diseases in humans and other organisms [3]. Opportunistic pathogens in aerosols enter the alveoli through the trachea and bronchi at a particle size about 2 μm, thereby causing serious infections [4]. Microbial aerosols not only infect animals directly, but also serve as carriers for antibiotic-resistant bacteria (ARBs) and antibiotic resistance genes (ARGs) [5]. In recent years, antimicrobial resistance (AMR) has received considerable attention as an emerging cause of a global public health crisis [6]. The treatment of bacterial infections is being

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increasingly hampered by the global emergence and spread of AMR, resulting in millions associated deaths [7]. In 2019, an estimated 4.95 million deaths were linked to ARBs infections globally, with 1.27 million of these deaths directly attributable to AMR [8]. Especially, fine particulate matter (PM_{2.5}) serves as a unique pathway for the environmental spread of ARGs and for the exposure of the general population through inhalation [9]. The spread of ARBs carried by aerosols exacerbates the difficulty and cost of treatment, highlighting the importance of understanding microbial aerosol properties for scientific and health purposes [10].

Aerosol and AMR are two major threats facing mankind in the 21st century [11,12], and have been extensively studied. In recent years, studies of microbial aerosols have mainly focused on indoor places such as livestock farms, sewage treatment plants, schools, hospitals, and biological laboratories etc. [11,13,14]. However, research on resistant microbial aerosols in pet hospitals remains scarce. Dogs and cats are some of the most popular pets in pet hospitals. They live with their owners and have close contact with their owners in daily life [12]. Pet hospitals, where pets, pet owners, and staff congregate, are potential hotspots for the spread of pathogenic microorganisms and ARBs. In addition, many antibiotics are used not only in humans but also in animals, thus increasing risk of cross-species transmission [15]. What is more, antibiotic resistant pathogens are present as a part of the inhaled bacteria, they might cause direct damage to worker, and pet owner health [16]. It has been reported that cat and dog fecal samples from pet hospitals exhibit a high abundance of ARGs, including those conferring resistance to various antibiotics such as aminoglycosides, tetracyclines, sulfonamides, lactams, macrolides, and chloramphenicols [5]. Studies have demonstrated that ARGs in environments such as manure can diffuse into the air and spread via airborne particles [12]. Therefore, the distribution of ARBs, AMR detection, and carried ARGs were studied to evaluate the risk of aerosol ARBs in pet hospitals to relevant personnel, in order to control the generation and dissemination of antibiotic-resistant microbial aerosols in pet hospitals.

In this research, microbial aerosols were collected from 5 pet hospitals in Changchun City using a six-stage Andersen Cascade Impactor (ACI) to assess the concentrations and size distribution of airborne bacteria [17]. The sampler simulated different parts of the respiratory tract according to aerodynamic principles and collected particles in six sections based on their aerodynamic diameter: >7.0 μm, 4.7–7.0 μm, 3.3–4.7 μm, 2.1–3.3 μm, 1.1–2.1 μm, and 0.65–1.1 μm, corresponding to particle size that may penetrate the nasal cavity, pharynx, trachea and primary bronchi, secondary bronchi, terminal bronchi, and alveoli, respectively (Table S1) [4,18,19]. Petri dishes can be placed at the position corresponding to each stage of the sampler to collect ARBs carried by the corresponding particle size. ARBs in various particle sizes were analyzed, and ARBs in PM_{2.5} (stage 6 and stage 5) were isolated, identified, and assessed for their minimum inhibitory concentration (MIC), and AMR properties. The expression of genes in the isolates was evaluated using Quantitative Polymerase Chain Reaction (qPCR) [14]. The results provide a foundation for risk analysis and prevention of aerosol resistance in pet hospitals.

2. Materials and methods

2.1. Sample collection

Air samples were collected using ACI from 5 pet hospitals (designated as A, B, C, D, and E) in different districts of Changchun, China, between July and September 2020. Each hospital's location and building characteristics are summarized in Table S2. Samples were collected at three locations within each hospital: a consulting room, an operating room, and an inpatient department. Sampling was conducted three times at each location, with doors and windows closed and no disinfection performed on the day of sampling. The temperature ranged from 25 to 30 °C, and humidity was maintained at 45–60% during sampling.

Brain-Heart Infusion Agar (BHIA) culture medium supplemented with cefotaxime (8 μg/mL) was used for collection and enrichment of airborne bacteria [13]. The prepared BHIA medium was introduced into the ACI according to international standards. Sampling was conducted for 20 min at a constant flow rate of 28.3 L/min, with the sampler positioned at a height of 1.2 m. Before and after each use, the sampler was sterilized with 75% ethanol [4]. Following sample collection, the plates were sealed and transported to the laboratory, where they were incubated at 37 °C for 24 h, and colony counts were performed. Due to variations in colonies morphology, single colonies were selected from stage 5 and 6, purified, amplified by PCR using 16 s rRNA primers, and sent to Sangon Bioengineering (Shanghai) Co., LTD for sequencing.

The concentration was calculated using the ventilation Eq. as follow:

$$CFU/m^3 = \frac{N1 + N2 + N3 + N4 + N5 + N6}{Q \times t} \times 1000$$

where, N_x represent number of bacteria in each tier of the sampler, Q represent gas flow rate (28.3 L/min), and t , represent sampling time (min).

2.2. Antimicrobial susceptibility testing

The isolates were underwent antimicrobial susceptibility testing using the microbroth dilution method following Clinical and Laboratory Standards Institute (CLSI, 2019) guidelines. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as quality control strains. Twelve antibiotics (listed in Table S3) were tested using Mueller-Hinton Broth (MHB) to prepare a 96-well gradient plate. Bacterial solutions were adjusted to a concentration of $1-2 \times 10^6$ CFU/mL, and equal volume of bacterial solution was added to each well and incubated at 37 °C for 16 h. The minimum inhibitory concentration was determined as the lowest concentration without visible bacterial growth. The experiment was repeated three times, and the final MIC was determined by considering the error of each result within a factor of 2.

2.3. DNA extraction and qPCR

Single colonies of the isolates were cultured in Luria-Bertani (LB) broth until reaching exponential phase, and subsequently diluted to a final concentration of $1-2 \times 10^6$ CFU/mL. Following this, 50 μL of each bacterial culture was mixed and subjected to a brief centrifugation to pellet the bacteria. DNA was extracted using the TIANNAMP Soil DNA Kit (TIANGEN, China) according to the manufacturer's instructions (Supporting information). The qPCR reaction system and conditions are shown in Table S4. Reaction conditions are shown in Fig.S2.

2.4. Statistical analysis

The average concentration of aerosols in each department and hospital was calculated from three samples collected. Graphical abstracts and figures were prepared using Microsoft Windows 6.1 and Origin 8.0, respectively. Statistical analysis and graphing were performed using Microsoft Excel 2019 and SPSS 22.0. Pearson's correlation coefficient (PC) > 0.6 was considered statistically significant for the correlation between species abundance and ARBs quantity. Fold change in gene expression was calculated as $2^{-\Delta Ct}$, where ΔCt represents the difference between the target gene and internal reference gene (16 s) Ct values [20].

3. Results

3.1. Concentration distribution of cefotaxime resistant bacteria

The concentration of cefotaxime-resistant bacteria at each hospital was determined by averaging the values obtained from the consulting

room, inpatient department, and operating room. Hospital A exhibited a concentration of 45 CFU/m³, Hospital B had 51 CFU/m³, Hospital C had 18 CFU/m³, and Hospital D had 86 CFU/m³ (Fig. 1a). Additionally, within each hospital, the concentration of cefotaxime-resistant strains varied across departments. Specifically, the consulting room of Hospital A had the highest concentration, while the inpatient departments of Hospitals B and D exhibited the highest content. Furthermore, the operating room of Hospital C displayed the highest concentration (Fig. 1b).

The mean concentration of cefotaxime-resistant bacteria in the inpatient department, operating room, and consulting room of the four hospitals indicated that the consulting room and the inpatient department had significantly concentration compared to the operating room ($p < 0.01$ and $p < 0.001$, respectively)(Fig. 1c). Cefotaxime-resistant bacteria were detected at all stages of the sampling process. Although no significant difference was observed in ARBs concentration among different stages, stages 3 and 5 exhibited the highest abundance of cefotaxime-resistant bacteria (Fig. 2c). In contrast, stage 6 samples consistently displayed the lowest ARBs concentration across hospitals and departments. Hospital E was excluded from the analysis due to insufficient isolate collection (Table S5, Fig. S3).

3.2. Isolation and identification of bacteria in stages 5 and 6

A total of 159 isolates were obtained from stages 5 and 6 (PM_{2.5}) of

the sampler (Fig. 3a). The detected genes (16 s rRNA) were deposited in NCBI GenBank under the following accession numbers: Hospital A, PP694171-PP694217; Hospital B, PP694218-PP694273; Hospital C, PP694330-PP694335; Hospital D, PP694341-PP694389. The isolates primarily comprised *Enterococcus* spp. (85 isolates, 51%), followed by *Staphylococcus* spp. (31 isolates), *Bacillus* spp. (22 isolates), *Pseudomonas* spp. (15 isolates), and minor occurrences of *E. coli*, *Acinetobacter*, *Flavobacterium*, *Stenotrophomonas maltophilia*, and *Elizabethkingia*. Variations in the distribution of ARBs genera were observed among hospitals, with one genus in hospital A and four genera in hospital B not found in other hospitals, and two genera (*Bacillus* spp. and *Enterococcus* spp.) prevalent in all hospitals (Fig. 3b). Additionally, differences in genus and quantity distribution of ARBs were noted among the four pet hospitals (Fig. 3c), with similarities observed between *Acinetobacter* in hospitals C and D, and distinct patterns observed in Hospital B compared to other hospitals.

3.3. Antimicrobial susceptibility testing of isolates

Antimicrobial susceptibility tests of 159 isolates showed that fourth-generation cephalosporins (cefquinome) exhibited MIC below 32 µg/mL, whereas third-generation cephalosporins (ceftriaxone and cefotaxime) showed MIC exceeding 512 µg/mL. The maximum MICs for tetracycline and doxycycline were 128 µg/mL and 32 µg/mL, respectively. Ciprofloxacin displayed a maximum MIC of 256 µg/mL, whereas

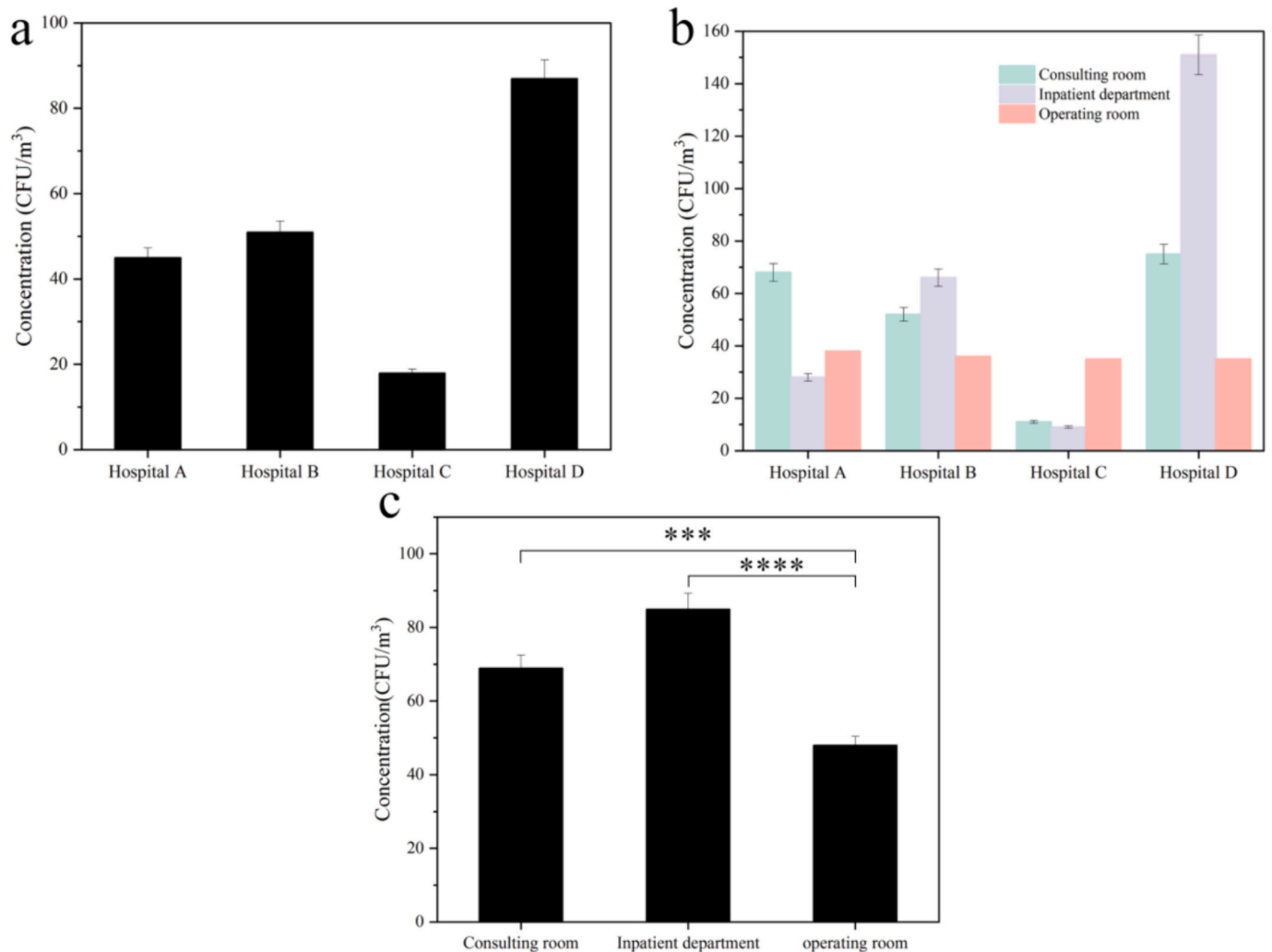


Fig. 1. (a) Concentration of ARBs in aerosols from different pet hospitals, (b) Concentration of ARBs in different departments within various pet hospitals, and (c) Overall concentration in different departments. *** $P \leq 0.01$, **** $P \leq 0.001$.

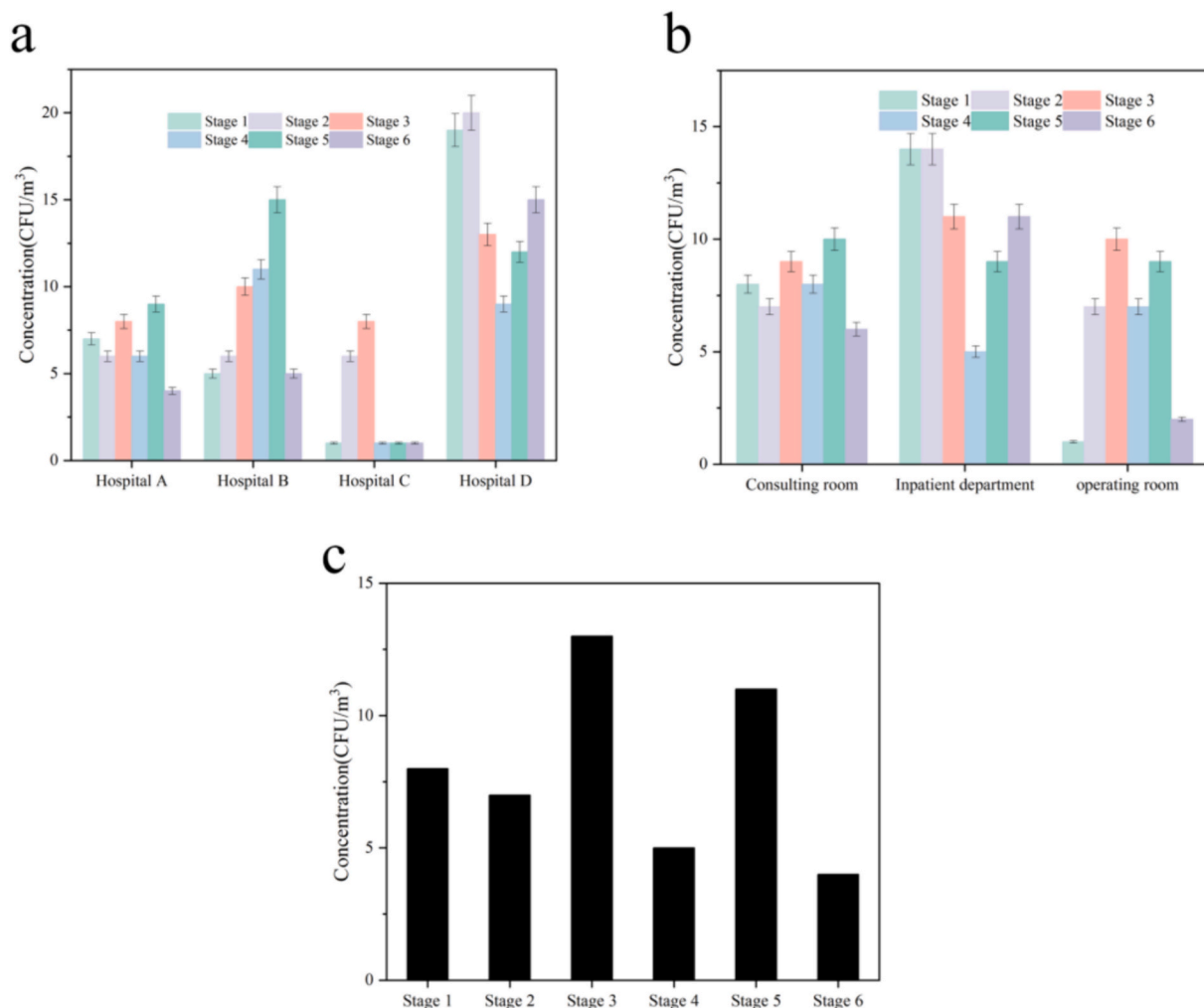


Fig. 2. (a) Concentration of ARBs in various stages within different pet hospitals, (b) Variation in ARB concentrations among different departments, and (c) Cumulative concentrations across different stages.

levofloxacin exhibited a maximum MIC of <32 $\mu\text{g}/\text{mL}$. The maximum MICs for the carbapenem antibiotics meropenem and imipenem were 512 $\mu\text{g}/\text{mL}$ and 128 $\mu\text{g}/\text{mL}$, respectively (Table S6). The analysis of resistance rate showed the highest resistant rate to tetracyclines (63%), while the lowest resistance rate was observed for cefquinoxime (27%). Resistance rates to imipenem, enrofloxacin, and doxycycline were 53%, 54%, and 52%, respectively (Fig. 4a).

Multidrug resistance (MDR) analysis revealed that 1 isolate (SO9-6) was resistant to 11 antibiotics, and 2 isolates (SO33-2 and SO33-5) to 10 antibiotics each. In total, 127 of the tested isolates (80%) demonstrated resistance to 3 or more antibiotics, and 32 isolates exhibited resistance to <3 antibiotics, while 7 isolates remained susceptible to all antibiotics. The distribution of MDR isolates from various hospitals is shown in Fig. 4b.

3.4. The relative expression of ARGs and transposon genes

Of the 279 genes analyzed (including ARGs and transposon genes, etc.), 165 were found to be expressed in the tested isolates, as summarized in Table S7. Among these 165 genes, the transposon gene *tnpA-07* exhibited the highest relative expression (Table S8). Notably, the top 10 expressed genes comprised 2 transposons, 3 tetracyclines-resistance genes, 2 MLSB (Macrolides, Lincosamides and Streptogramins B) genes, and 3 aminoglycoside genes. The detected genes have been shown to be associated with resistance to various antibiotics, including

carbapenems, penicillins, cephalosporins, quinolones, aminoglycosides, macrolides, and even vancomycin.

The relative expression and detection number of various genes are presented in Table S9. Among these genes, the highest relative expression was found in mobile genetic elements (MGEs) (43.72%), followed by aminoglycoside, tetracyclines, MLSB, β -lactam, FCA (Fluoroquinolone, Quinolone, Florfenicol, Chloramphenicol and Amide alcohols), and sulfonamide, etc. (Fig. 5). The expression frequency of other/efflux was only 0.66%. The relative expression of vancomycin resistance genes was comparatively low ($5.77 \times 10^{-4}\%$).

The detected expression genes included 35 MLSB genes, 31 tetracyclines genes, 27 β -lactam and aminoglycoside genes, 20 FAC genes, 10 vancomycin genes, 7 MGEs, 3 sulfonamide genes, and 5 other genes (Fig. 6).

4. Discussion

Historically, third-generation cephalosporin antimicrobials have been extensively utilized in both human and veterinary medicine for treating infections [21]. However, resistance to third-generation cephalosporin now poses a substantial threat to both human and animal health globally [22]. Therefore, this study employed cefotaxime to directly evaluate the presence of cephalosporin resistance in microbial aerosols, thereby reflecting the levels of ARBs in pet hospital environments.

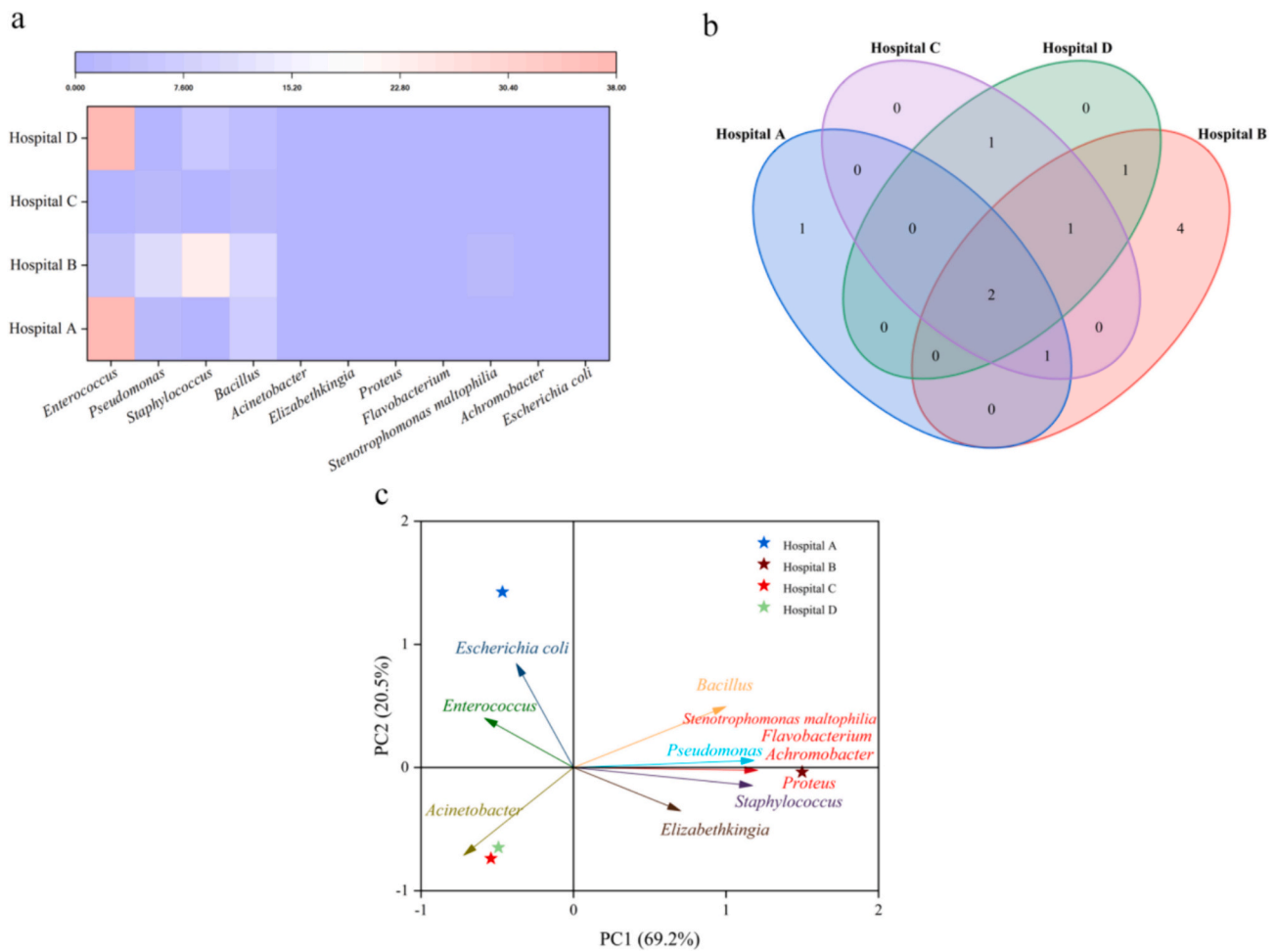


Fig. 3. Isolation of ARBs from PM_{2.5} in 4 pet hospitals. (a) Assessment of ARB distribution across four pet hospitals. (b) Analysis of the distribution of various bacterial genera within each hospital. (c) Principal component analysis (PCA) of the number of ARBs species abundance across different hospitals.

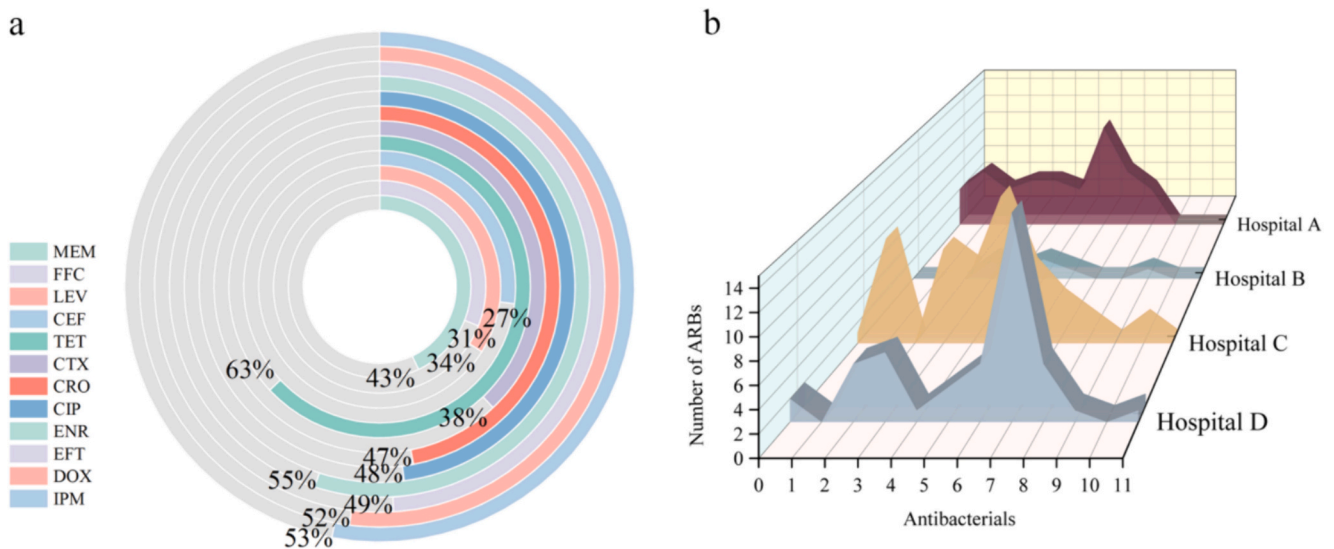


Fig. 4. Susceptibility analysis of ARBs to 12 antimicrobial agents. (a) Assessment of resistance rates of ARBs to different antibiotics. (b) Analysis of MDR patterns of ARBs to multiple antibiotics. Abbreviations: MEM (meropenem), FFC (florfenicol), LEV (levofloxacin), CEF (cefquinome), TET (tetracycline), CTX (cefotaxime), EFT (ceftiofur), CRO (ceftriaxone), CIP (ciprofloxacin), ENR (enrofloxacin), DOX (doxycycline), IPM (imipenem).

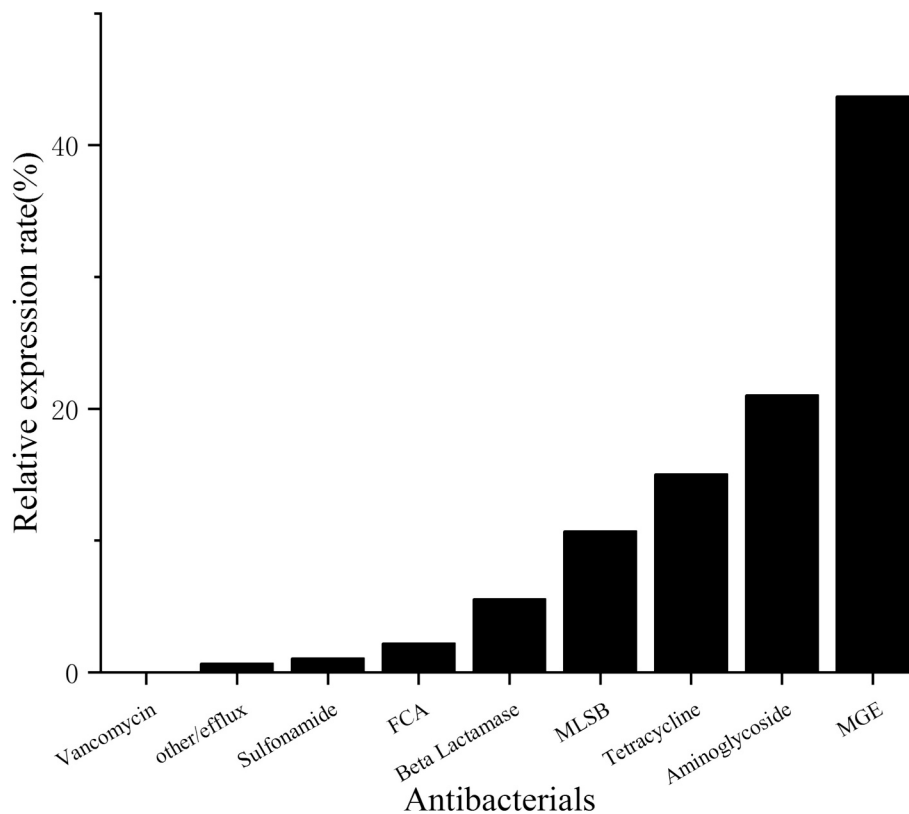


Fig. 5. The Relative Expression Percentage of genes. The highest expression was observed for MGE at 43.72%, followed by aminoglycoside (21.03%), tetracyclines (15.03%), MLSB (10.70%), β -lactam (5.58%), FCA (2.20%), sulfonamide (1.06%) and other resistance genes (0.66%). The relative expression of vancomycin resistance genes was comparatively low (5.77×10^{-4} %).

Previous studies indicate that indoor microbial compositions can be significantly influenced by human occupants and their activities [23–25]. Non-human occupants, including pets [26–28], and household insects [29] also contribute to indoor microbial diversity. Furthermore, source apportionment of airborne microorganisms collected in a pet-friendly office revealed that 40% of them originate from humans, 30% from outdoors, and 30% from dogs [30]. Additionally, transmission of ARBs into the air through animal feces has been reported [5,31]. Indoor microbial compositions are not only influenced by human and animals in the room, but may also be related to the building function and age. Therefore, variability in bacterial concentrations and genera among hospitals may be attributed to differences in personnel and animal mobility, geographical location, as well as hospital's operational history [32]. Although literature linking indoor bacterial concentrations to building function and age is limited [33], our study suggests a potential association between ARBs concentration and hospital service time. For example, Hospital E has only been operating for 2 months, and the concentration of ARBs were very low. It is speculated that the low number of ARBs may be due to the short operation period, but this speculation needs further research. Interestingly, lower ARBs concentrations in certain hospitals, such as the inpatient department of Hospital A, may be linked to specific disinfection practices. Conversely, higher ARBs concentrations in the operating room of Hospital C may be associated with surgeries throughout the day, as observed in our return visit findings [34]. Different from the findings of Bouillard et al., the most common species in the air of a healthy office building were *Micrococcus* spp., *Staphylococcus* spp., and *Streptococcaceae* spp. [35]. In a pet hospital setting, where pets and humans congregate, airborne microbial sources may vary. Our findings revealed *Enterococcus* spp. as the predominant species, possibly due to natural resistance to cephalosporins or predominant presence in pet-associated environments [5].

ACI samplers correspond to different segments of the human lung,

with stages 1–4 corresponding to diameters capable of entering the upper respiratory tract. Stages 5 and 6 correspond to diameters that can enter the lower respiratory tract and also fall within the PM_{2.5} range [4,36,37]. ARBs were distributed in each stage in this study, with the 3rd and 5th levels (3.3–4.7 μ m and 1.1–2.1 μ m, respectively) exhibiting the highest abundance in sampling, suggesting their ability to penetrate both the upper and lower respiratory tract. This penetration was particularly notable in stages 5 and 6, which represent particle sizes capable of entering the lower respiratory tract.

In this research, tetracycline and enrofloxacin exhibited the highest resistance rates of 63% and 55%, respectively. The resistance to tetracyclines and enrofloxacin, commonly used for the treatment of infectious diseases both in humans and animals, poses a serious issue in China as well as worldwide [12,13,38,39]. Additionally, the highest level of carbapenem resistance among isolate exceeded 512 μ g/mL. Since carbapenems are not allowed for use in animals in China, the emergence of carbapenem resistance underscores the broader issue of AMR dissemination [15,40]. Moreover, the resistance of these ARBs can be acquired through lateral transfer of ARGs from other bacteria or the environment, facilitated by high expression of MGEs (2 of the top 10 genes exhibiting the highest relative expression were MGEs), including plasmids, integrons, transposons, and phages [41–45]. Among them, *tnpA* is a factor facilitating the transfer of ARGs encoding transposases [38], the activity of *tnpA* may be influenced by the substantial volume of human traffic [16]. The relative expression of 279 genes showed that *tnpA* had the highest relative expression, which may be the reason for the high concentration of ARBs carried by aerosol in pet hospitals and the serious AMR. Moreover, the diverse and complex AMR landscape in pet hospital aerosols is consistent with the detection results of ARGs in the smoggy weather of Beijing [46]. Therefore, it is urgent to control the generation of antimicrobial resistant bacteria in the aerosol of pet hospitals.

To control the spread of ARBs in the air, the concentration of

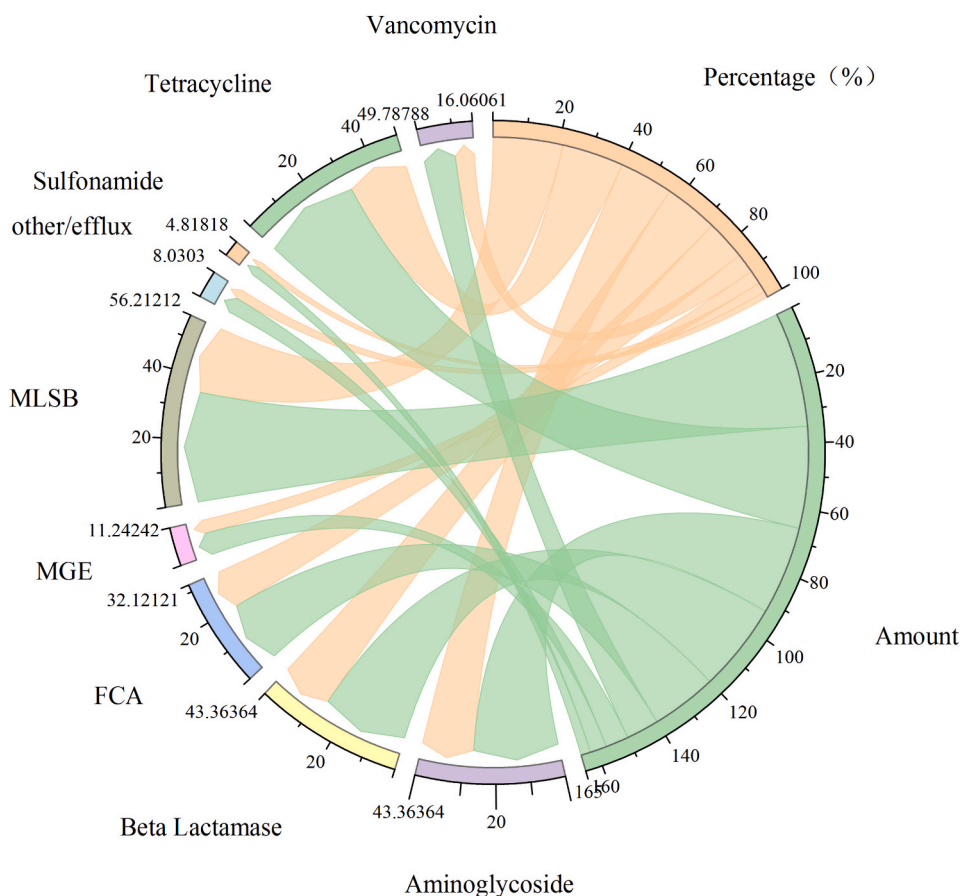


Fig. 6. Circos distribution of genes. The highest detection rate was observed for MLSB genes with 35 occurrences (21.21%), followed by tetracyclines resistance genes with 31 occurrences (18.79%). Aminoglycosides and β -lactam resistance genes each had 27 occurrences (16.36%), while FCA had 20 occurrences (12.12%); Vancomycin resistance genes were detected 7 times (4.24%), MGE were detected 7 times (4.24%), and sulfonamides resistance genes were detected 3 times (1.81%). Five other resistance genes were detected, each with 5 occurrences (3.03%).

airborne particles must be reduced. The reason for this is not clear, but it may be related to the fact that these particles provide a rich environment with sufficient water and nutrients for a significant duration, allowing the antibiotic-resistant plasmids. Active mobile elements likely play a significant role in facilitating resistance gene dissemination within aerosols [4,44]. In conclusion, understanding the dynamics of ARBs dissemination in pet hospital environments is imperative for implementing effective control measures to combat AMR propagation.

5. Conclusion

Our findings indicate a significant presence of ARBs capable of directly entering the lower respiratory tract in air samples collected from pet hospitals. These ARBs exhibit high levels of antimicrobial resistance, with the majority displaying MDR. Moreover, the expression of MGEs is notably elevated in these isolate. Given that ARBs in $PM_{2.5}$ aerosols heighten the risk of AMR transmission and infection in humans, it is imperative to address this issue by implementing stringent control the use of antibacterial antibiotic.

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CRedit authorship contribution statement

Dao Mi Zhu: Writing – original draft. **Ya Song Yan:** Formal analysis. **Hao Wang:** Formal analysis. **Inam:** Writing - original draft. **Yun Hang Gao:** Methodology. **Gong Mei Li:** Funding acquisition. **Guo Dong Mu:** Methodology. **Hui feng Dong, Yuan Li:** Investigation. **Ding kuo Liu:** Project administration. **Hong Xia Ma:** Conceptualization, Funding acquisition. **Ling Cong Kong:** Conceptualization, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.onehlt.2024.100765>.

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