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Original Article

Monitoring and analysis of pathogenic microorganisms and resistance genes

Min Zhang[1], Guimei Kong[1], Yang Li[2], Yuehan Li[1], Yan Xu[2]*

[1] *Department of Clinical Medicine, Yangzhou University, Yangzhou, Jiangsu 225000, China*
[2] *Department of Respiratory and Critical Care Medicine, The Affiliated Hospital of Yangzhou University, Yangzhou University, Yangzhou, Jiangsu 225000, China*

Article Info

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Abstract

The objectives are to improve the rapid identification method of microbial risk and cut off the route of transmission of resistance genes. When new pathogenic microorganisms are found, intervention can be carried out as early as possible to identify the risk of potential pathogen transmission, and timely cut off the transmission route. Hospital air samples were collected to analyse the distribution of environmental pathogenic microorganisms and the characteristics of ARGs resistance genes. The air samples were collected from 12 sampling sites in the Affiliated Hospital of Yangzhou University. In the infusion room, general ward and intensive care unit, no significant difference was found in microorganisms, and no significant difference was found in microbial resistance genes. There were some differences in resistance genes between east and west districts. Combined with the detection of pathogenic microorganisms and resistance genes in our hospital, it is necessary to improve the daily disinfection measures such as air conditioning and fresh air equipment, cut off the infection route, block the transmission of resistance genes, and monitor pathogens and resistance genes in airborne diseases.

Keywords: Antibiosis, Drug resistance, Microbiology, Nosocomial infection, Resistance genes

1. Introduction

The outbreak of the coronavirus disease 2019 has made the prevention and control of explosive infectious diseases a top priority. The development of new methods and technologies for accurate identification of pathogenic microorganisms and rapid and effective screening of new infectious diseases of unknown causes is particularly important for risk management and control of public health events. At the end of 2019, mNGS became the main detection method for the detection of unknown pathogens, such as novel coronavirus. At present, the diagnosis rate of pathogens has been less than 30 % in most hospitals in China. Even in tertiary hospitals, the detection rate of pathogens is very low. Because the positive frequency of conventional specimen culture is very low, the traditional pathogen identification methods such as haemoculture and sputum cultivation are gradually replaced by immunological diagnosis, nucleic acid amplification and metagenomics based on high-throughput sequencing [1]. Now immunostrip, isothermal amplification and fluorescence quantitative PCR have made rapid progress in etiological detection with the main advantage of low price. But these tend to be specific to a particular class, or a few key pathogens.

Metagenomic technology still has irreplaceable advantages. Many studies [2–6] have shown that metagenomics (mNGS) is the most advanced etiological detection method at present, which can simultaneously compare multiple microbial pathogens, indicate the relative proportion of dominant pathogens, and provide additional diagnostic information, such as virulence and resistance genes of pathogenic bacteria. It is also an advantageous method for the accurate identification of pathogenic microorganisms in the discovery of unexplained emerging infectious diseases, which can assist in the formulation of antimicrobial treatment plans. However, the cost and detection period greatly restrict the detection of pathogens by metagenomics.

1.1. The main reason for the high cost of sequencing is the contamination of human.

DNA in the host. The length of the human genome is much longer than that of bacteria. In biological samples with low bacterial concentration, contamination of a small number of host cells will introduce a large number of host DNA, resulting in a sharp increase in invalid sequences. The detection cost can be greatly reduced by pretreatment

Monitoring and analysis of pathogenic

Cell. Mol. Biol. 2024, 70(4): 181-190

before sample extraction. The use of a Host Zero kit to remove human DNA can reduce the detection cost of DNA sequences and improve the sensitivity of pathogen detection [7]. However, RNA is not limited to the contamination of host human DNA. For the detection of RNA viruses, RNA sequencing is used, and the problem of host contamination is not serious.

1.2. Another problem that restricts the clinical development of metagenomics (mNGS) is the long detection period.

At present, RT-PCR and corresponding virus detection reagents are no longer technically restricted. The sampler for specimen extraction can greatly improve the sampling rate at the expense of a small part of the rejection rate. Rapid RT-PCR analysis is possible, and timely reporting through the Internet, for early warning of possible outbreaks of infectious diseases.

1.3. The biological contamination of the hospital environment can cause outbreaks and epidemics of a series of infectious diseases.

Airborne pathogenic microorganisms can cause the epidemic of infectious diseases through airborne transmission. There are a large number of microorganisms in the air. They usually attach to particles of a certain size and exist in the form of aerosols, such as dust, droplet nuclei, etc., mainly invading the body through the respiratory tract, and causing respiratory tract infection. It has been shown that aerosol transmission is a non-negligible route of transmission of *Acinetobacter baumannii* during open-airway aspiration and is an important source of environmental contamination of *A. baumannii* in the ICU [8]. A prospective study abroad has shown that the environment is a possible transmission route of *A. baumannii* [9]. Most of these pathogenic bacteria and opportunistic pathogens have been isolated from clinical specimens and are important pathogens of nosocomial infection. In the past, the transmission of microbial aerosols in hospital environment was mainly confirmed by the natural sedimentation method of petri dishes to detect the change in bacterial concentration, bacterial resistance and the content of some pathogenic bacteria. However, it has not been proved that the origin and homology of microbial aerosols isolated from the hospital ward environment, the internal and external environment of the outpatient room, and the content of aerosol microorganisms were not determined by the international universal sampler. It has limitations. At present, the detection of drug-resistant bacteria used in nosocomial infections often stays at the bacterial level, with less molecular epidemiological intervention and less research on resistance genes. Respiratory tract infections are often the main site of infection in nosocomial infections, where aerosol transmission and contact transmission are predominant. In the field of anti-infection, our country should take into account the actual situation in our country, because of the national and regional differences in the distribution of pathogenic microorganisms and drug resistance, we cannot copy foreign guidelines. In Sweden, for example, the rate of multi-drug-resistant infections is at a low level [10]. On the contrary, in China, the first multi-center epidemiological survey of hospital-acquired pneumonia in China with a large sample size was published by Professor You-ning Liu in 2012, and the results showed

that the pathogens of hospital-acquired pneumonia in China and other Asian countries were mainly multidrug resistant non-fermentative bacteria, which was completely different from the 2005 ATS guidelines [11]. Even the survey results of nosocomial infection in our country are not completely consistent, and the incidence rate varies greatly among different departments of the same hospital.

The prevention and control of nosocomial infection is not only paid attention to because of the COVID-19 epidemic but also the infection of drug-resistant bacteria is regarded as one of the three major infectious diseases in daily hospital management. There is no corresponding industry standard on how to prevent nosocomial infections in China. Cutting off the route of infection and blocking the transmission of resistance genes is particularly important for the monitoring of pathogens and resistance genes in airborne diseases, which has a positive significance for the control of nosocomial infection. Many bacteria implement their resistance mechanism through antibiotic resistance genes (ARGs). Bacteria themselves naturally have resistance genes, and strains containing resistance genes survive and proliferate after antimicrobial drugs have eliminated normally sensitive strains. Under the screening of antimicrobial agents, the antibiotic resistance genes in the clinic are closely related to those found in the environment. The environment is a reservoir of resistance genes. In the process of transmission of resistance genes, airborne microorganisms and ARGs pollution have attracted more and more attention. Due to the particularity of hospitals' functions and service objects, the use of medical antibiotics is essentially short-term high-dose exposure, so there may be more serious contamination problems of pathogens and ARGs in hospitals.

In the current situation, pathogens are spatially and temporally transmitted while continuously mutating, so it is necessary to monitor both pathogens and resistance genes. Therefore, the detection of pathogens and resistance genes in aerosols between different departments within the hospital and the determination of homology can assess the hazard of microbial aerosols in the hospital environment and the pathways of airborne transmission of drug-resistant bacteria.

2. Materials and methods
2.1. Sample collection and preprocessing method

Sampler technique: Using a sampler with a high flow rate (air flow rate > 100L/min), which has high interception efficiency, safe transfer of samples after sampling, and quick and simple detection and analysis for sampling. It can intercept fine virus particles and realize the collection and detection of viruses and other pathogens in the air medium. At the same time, it can accelerate the collection speed and improve the detection efficiency. We can use it to realize automatic sampling in a large spatial range in densely populated areas such as hospitals.

The use of a water-soluble gelatin filter membrane makes it possible to collect viruses in the air medium. Technical parameters of gelatin filter membrane (with water solubility): pore size: 1-3μm, membrane diameter: 80mm, thickness: 200-450μm, membrane temperature: 70°C, membrane moisture content: 46 % to 49 %, gas velocity: 2.7l / min per cm_2, working pressure difference: 0.7psi, effective filtration area: $40cm_2$, rejection rate: a) Bacillus subtilis: > 99 % b) Phage: > 95 %. The membrane

Monitoring and analysis of pathogenic

Cell. Mol. Biol. 2024, 70(4): 181-190

was packaged independently and sterilized by irradiation. This enables the detection of pathogens such as viruses. Refrigerate and seal the gelatin membrane to avoid infection during transportation.

The rapid nucleic acid extraction, purification and concentration of air microbial samples and risk microbial identification report were realized. Pretreatment technology is used to remove human DNA before nucleic acid extraction, which greatly reduces the cost of sequencing.

2.2. Collection of pathogenic microorganisms in the hospital environment

The Affiliated Hospital of Yangzhou University was selected as the sampling point. The hospital has two hospital districts, which are divided into eastern and western hospital districts, about 6 km apart. A total of 12 sites were sampled, they were the western infusion room (specimen number W1), pulmonary and critical care medicine in the western district (specimen number W2), the ICU in the western district (intensive care unit, specimen number W3), department of nephrology in the western district, department of gastroenterology in the western district, department of neurosurgery in the western district, the eastern infusion room (specimen number E1), pulmonary and critical care medicine in the eastern district (specimen number E2), the ICU in the eastern district (specimen number E3), department of endocrinology in the eastern district, department of cardiovascular medicine in the eastern district and department of urology in the eastern district. The sampling points were designed and numbered in the outpatient infusion hall, general inpatient ward and intensive care unit of the hospital, and a high flow rate sampler was used to collect samples for 24 hours.

2.3. Sample extraction and detection

The CTAB method was used to extract genomic DNA from the collected samples. The concentration, integrity and purity of the DNA were measured by Agilent 2100.

2.4. Bioinformatics analysis
2.4.1. Data quality control and de-host sequences

By using the high-throughput sequencing platform Illumina Novaseq for metagenomic sequencing, the raw data of the bacteria, fungi, and viruses in the samples were collected. In order to ensure the reliability of the Data, Kneaddata software was used to preprocess the Raw sequencing data, remove splice sequences from the raw data, remove low-quality sequences, and remove sequences with final length of less than 50bp. Considering the possible host contamination of the sample, it was necessary to align the Clean Data to the host genome. Bowtie2 software was used by default to filter the sequences from the host and obtain valid sequences for subsequent analysis. Finally, FastQC was used to detect the rationality and effect of quality control [12–14].

2.4.2. Taxonomy annotation

The number of species in the samples was calculated using Kraken2 and self-developed microbial nucleic acid databases (NCBI NT nucleic acid database and RefSeq whole genome database). Bracken was used to forecast the relative abundance of the species in the samples.

2.4.3. Resistance gene annotation

The sequences of each sample after quality control and de-hosting were compared with the CARD database using DIAMOND software [15], the sequences that failed the comparison were filtered out, and the relative abundance of antibiotic resistance genes in each sample was obtained based on the results of the comparison.

3. Results
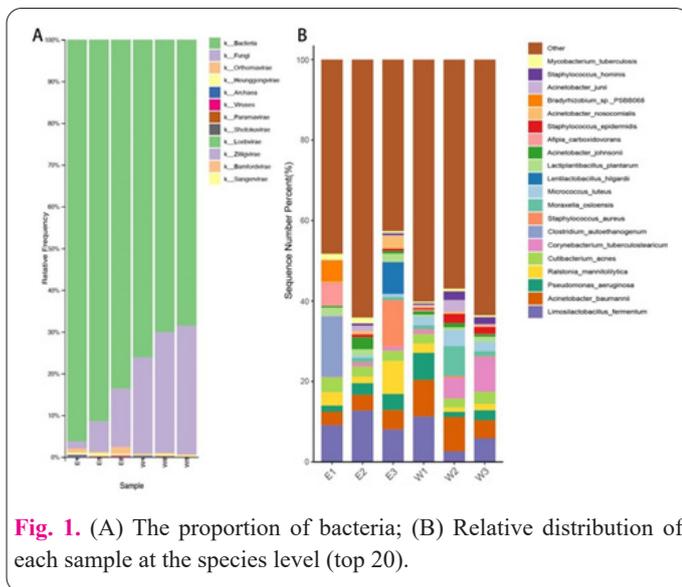### 3.1. Analysis of pathogenic microorganism composition in hospital air medium

To study the species composition and diversity information of the sample, we annotate and classify all the valid sequences of all samples and count the proportion of the number of sequences on each sample at a total of seven classification levels (Kingdom, Phylum, Class, Order, Family, Genus, Species) to the total number of sequences, which can effectively evaluate the species annotation resolution of the sample.

In the sample, the species we detected were Archaea (34410), Bacteria (20686160), Bamfordvirae (1098), Fungi (4624220), Heunggongvirae (123546), Loebvirae (1636), Orthornavirae (123368), Pararnavirae (8040), Sangervirae (104), Shotokuvirae (7793), Viruses (17664), Zilligvirae (1581). The proportion of species was Archaea (0.13%), Bacteria (80.71%), Bamfordvirae (0.00%), Fungi (18.04%), Heunggongvirae (0.48%), Loebvirae (0.01%), Orthornavirae (0.48%), Pararnavirae (0.03%), Sangervirae (0.00%), Shotokuvirae (0.03%), Viruses (0.07%), Zilligvirae (0.01%). The top five species detected were: Bacteria, Fungi, Orthornavirae, Heunggongvirae, and Archaea.

To understand the situation of pathogenic microorganisms in the air of the hospital, we selected a total of six representative sampling sites in the east and west areas of the Affiliated Hospital of Yangzhou University. We selected the infusion room (western district, specimen number W1), pulmonary and critical care medicine (western district, specimen number W2), ICU (western district, specimen number W3), infusion room hall (eastern district, specimen number E1), pulmonary and critical care medicine (eastern district, specimen number E2), ICU (eastern district, specimen number E3) as sampling points to analyze the air in different places of the hospital. The proportion of bacteria in the air of each sampling site is shown in Figure 1A.

As can be seen from the figure, bacteria accounted for a high proportion of the air samples, with E1 bacteria accounting for 96.266%, E2 bacteria for 83.639%; E3 bacteria for 91.473%; W1 bacteria for 76.113%; W2 bacteria for 70.084%; and W3 bacteria for 68.546%. Therefore, we mainly studied the bacteria in the collected air samples from the hospital.

As in Figure 1B, the horizontal coordinate is the sample name, the vertical coordinate (Sequence Number Percent) indicates the ratio of the number of sequences annotated to that species level to the total annotated data, and the color order of the histogram from the top to bottom corresponds to the color order of the legend on the right. Sequences that are not annotated at the species level are categorized as unclassified. The 20 most dominant species are shown in the legend, and the remaining species with lower relative abundance are categorized as Other in the figure. The dominant bacteria in the eastern infusion room were: *Cuti-*

Monitoring and analysis of pathogenic

Cell. Mol. Biol. 2024, 70(4): 181-190

Fig. 1. (A) The proportion of bacteria; (B) Relative distribution of each sample at the species level (top 20).

bacterium acnes (3.7%), *A. baumannii* (3.3%), *Ralstonia mannitolilytica* (3.3%), and *Pseudomonas aeruginosa* (1.5%). The dominant bacteria of pulmonary and critical care medicine in the eastern district were *A. baumannii* (3.9%), *Acinetobacter johnsonii* (2.9%), *P. aeruginosa* (2.8%), and *C. acnes* (2.5%). The dominant bacteria of the ICU in the eastern district were *Staphylococcus aureus* (11.5%), *R. mannitolilytica* (8.2%), *A. baumannii* (4.8%), and *P. aeruginosa* (4.0%). The dominant bacteria of the western infusion room were *A. baumannii* (9.1%), *P. aeruginosa* (6.7%), *C. acnes* (2.4%), and *R. mannitolilytica* (2.3%). The dominant bacteria of pulmonary and critical care medicine in the western district were *A. baumannii* (8.5%), *Moraxella osloensis* (7.5%), *Corynebacterium tuberculostearicum* (5.1%), *Micrococcus luteus* (4.0%). The dominant bacteria of the ICU in the western district were *C. tuberculostearicum* (8.6%), *A. baumannii* (4.5%), *C. acnes* (3.0%), and *P. aeruginosa* (2.4%).
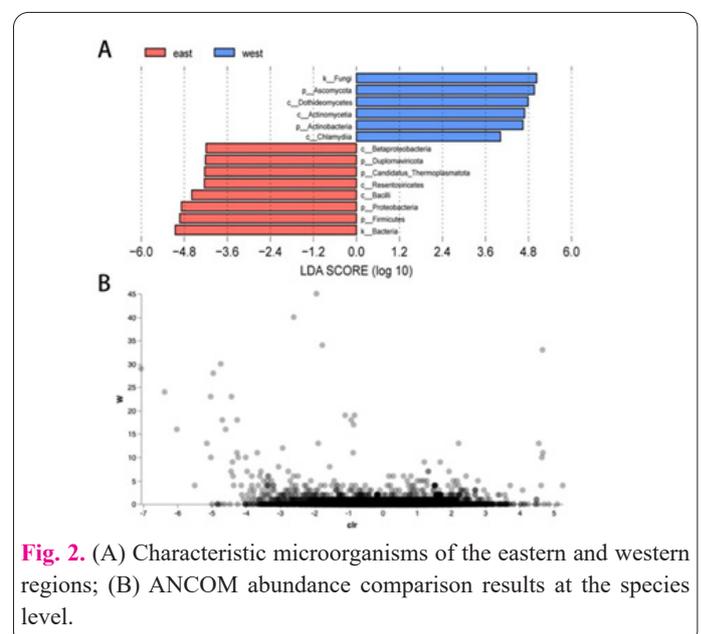
Firstly, we compared the pathogenic microorganisms in the infusion room, general ward and intensive care unit, and performed LEfSe analysis (LDA Effect Size analysis is an analytical tool for the discovery and interpretation of biomarkers for high-dimensional data, including genes, pathways, and taxa. It can be compared in two or more groups. It emphasizes the statistical significance and biological relevance, and can find biomarkers with statistical differences between groups.), find the characteristic microorganisms (microorganisms with LDA > 2) [16] of each group, which were a higher abundance of microorganisms in this group than other groups. We did not find significantly different microorganisms in the infusion room, general ward, and intensive care unit.

To study the similarity between different samples, we perform cluster analysis on the samples to construct clusters of samples. By default, the top 20 absolute abundance of species are selected, and the sample clustering is realized according to the species composition of the samples. In order to investigate the similarities or differences between different samples or groups, we also cluster according to the distribution of species abundance in each sample to find the aggregation law of species or samples [16, 17]. It can be seen from the results of cluster analysis that among the three groups, the relative abundance of bacilli in the eastern ICU was the highest, and the rela-

tive abundance of actinomycetia in the western ICU was the highest. In the eastern ward of pulmonary and critical care medicine, the relative abundance of bacilli was the highest. In the western ward of pulmonary and critical care medicine, the relative abundance of gamma-proteobacteria (γ-proteobacteria) was the highest. The relative abundance of alpha-proteobacteria (α-proteobacteria) in the eastern infusion room hall was the highest, and the relative abundance of gamma-proteobacteria (γ-proteobacteria) in the western infusion room was the highest.

There was a comparison of microbes in the east and west areas of the hospital. Figure 2A shows the characteristic microorganisms of the eastern and western regions, where each transverse column represents a species, the length of the column corresponds to the LDA value (the higher the LDA value, the greater the difference), and the color of the column corresponds to the species of the characteristic microorganism (the abundance in the corresponding group is relatively high) in the corresponding group. According to the comparison, the largest difference in the western region is Fungi, followed by Ascomycota, the third is Dothideomycetes, and then Actinomycetes; the largest difference in the eastern region was Bacteria, followed by Firmicutes, then Proteobacteria, and finally Bacilli.

ANCOM [18] (Analysis of the composition of microbiomes) is another analytical method to compare the significant differences of species between groups in microbiomics data. ANCOM analysis does not rely on the assumption of distribution of the data and addresses the limitations imposed by the relative abundance analysis in other methods, thus being able to effectively reduce the results of False positives. As shown in Figure 2B, W-value is a statistic (similar to F-value, t-value) to measure the significance of the difference between groups. The higher the W-value, the higher the significance of the difference between groups. Each point in the figure represents a comparative species, with the ordinate representing the W-value and the horizontal clr value representing the degree of variation in sample abundance between groups, and the higher the absolute value of the number, the greater the relative abundance difference. Therefore, the closer the point in the figure is to the upper right corner (or the upper

Fig. 2. (A) Characteristic microorganisms of the eastern and western regions; (B) ANCOM abundance comparison results at the species level.

left corner), the more significant the difference between the species and other species (species near the horizontal axis 0). We can learn that there is no significant difference in the performance of species at each taxonomic level between groups, which means that there is no significant difference in pathogenic microorganisms between the eastern and western regions.

3.2. Characterization of ARGs in hospital environment

Bacterial antibiotic resistance is the resistance of bacteria to specific antibiotics through gene mutation or transfer from environmental organisms at the gene level under the premise of large-scale antibiotic use by humans to control bacterial growth. These mutations make the target of antibiotic action change or can no longer exert action on its target. The acquisition of resistance usually involves complex interactions between gene/mobile DNA elements and their bacterial hosts.

Based on the relative abundance table of resistance genes, the content and percentage of ARO in each sample were calculated. The results of the top 20 ARO with the highest abundance were shown in Figure 3A and compared with the CARD database. The CARD [19, 20] database is currently the most widely used resistance gene database, including 3997 resistance gene classifications, and provides online search interfaces for each classification name with databases such as PDB and NCBI for subsequent analysis. According to the comparison results, the result of the top five of each sample were that E1: other, catI, Plasmid-encoded cat (pp-cat), ARO: 3004459, APH(3')-IIIa; E2: other, Bado_rpoB_RIF, APH(3')-IIIa, tet(O), rpoB2; E3: other, ErmC, Erm(33), ErmT, PC1 beta-lactamase (blaZ); W1: other, rpoB2, Bado_rpoB_RIF, mtrA, qacH; W2: other, ErmC, lnuA, rpoB2, Erm(33); W3: other, lnuA, rpoB2, ErmX, ErmC. The individual genes in the samples (top 10 in abundance) are screened and a Circos plot is drawn, which can show the proportion of each gene in each sample. As in Figure 3B, the left half-circle shows the ten resistance genes with the highest abundance, and within each resistance gene, different colors represent the proportion of different sample sources; the right half-circle shows the samples, and different colors within the samples represent the proportion of different resistance genes.
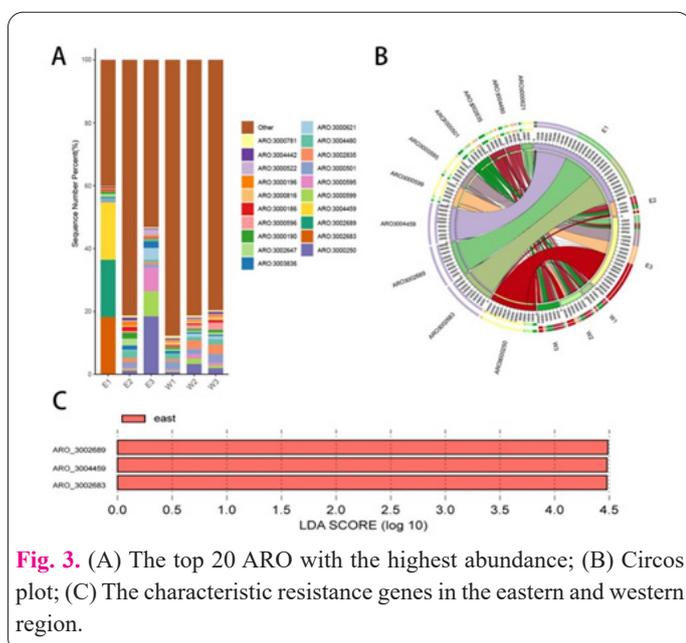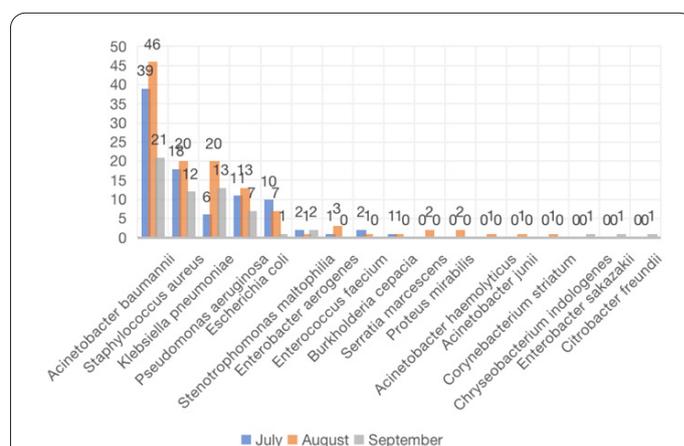
According to the CARD database, the main resistance gene in the eastern infusion room was catI (resistance gene of *Escherichia coli*). The main resistance gene in pulmonary and critical care medicine in the eastern district was APH (3') -IIIa (related to aminoglycoside antibiotics). The main resistance gene in the ICU in the eastern district was ErmC (mainly related to macrolide antibiotics). The main resistance gene in the western infusion room was mtrA (related to macrolide antibiotics and penem). The main resistance gene in pulmonary and critical care medicine in the western district was ErmC (mainly related to macrolide antibiotics). The main resistance genes in the ICU in the western district were ErmX and ErmC (both related to macrolide antibiotics).

Combined with the grouping information, LEfSe was used to analyze the microbial resistance genes in the infusion room, inpatient department, and intensive care unit, and no significant difference was found between the three groups. Then we compared the resistance genes in the eastern and western regions (the LDA value was selected as 4). As shown in Figure 3C, the characteristic resistance genes in the eastern region were ARO_3002689(pp-cat, Plasmid-encoded cat, or pp-cat, is a plasmid-encoded variant of the cat gene found in *Photobacterium damselae* subsp. piscicida.), ARO_3004459, and ARO_3002683(catI, catI is a chromosome and transposon-encoded variant of the cat gene found in *E. coli* and *A. baumannii*.).

3.3. Comparison with the results of laboratory culture of drug-resistant bacteria in the hospital in the third quarter of 2022

In the third quarter of 2022 (July, August, and September), 205 infected patients with 268 drug-resistant strains were monitored among 24060 hospitalized patients. Among them, 268 strains (Figure 4) of multidrug-resistant/targeted drug-resistant strains were 106 strains of *A. baumannii*, 50 strains of *S. aureus*, 39 strains of *Klebsiella pneumoniae*, 31 strains of *P. aeruginosa*, 18 strains of *E. coli*, 5 strains of *Stenotrophomonas maltophilia*, 4 strains of *Enterobacter aerogenes*, 3 strains of *Enterococcus faecium*, 2 strains of *Burkholderia cepacia*, *Serratia marcescens*, *Proteus mirabilis*, 1 strain of *Acinetobacter haemolyticus*, *Acinetobacter junii*, *Corynebacterium striatum*, *Chryseobacterium indologenes*, *Enterobacter sakazakii*, *Citrobacter freundii*. This is roughly similar to the composition of pathogenic microorganisms detected in air

Fig. 3. (A) The top 20 ARO with the highest abundance; (B) Circos plot; (C) The characteristic resistance genes in the eastern and western region.

Fig. 4. The composition of 268 multidrug-resistant/targeted drug-resistant strains.

Monitoring and analysis of pathogenic

Cell. Mol. Biol. 2024, 70(4): 181-190

samples. Considering the consistency of air samples and laboratory culture results of drug-resistant bacteria in hospitals. Therefore, the results of air samples and hospital drug-resistant bacteria laboratory culture are consistent. There were more kinds of pathogenic microorganisms in air samples, and the positive rate of routine drug-resistant bacteria laboratory culture was low, so air samples can better detect pathogenic bacteria in the air.

3.4. Comparison of pathogenic bacteria in Western ICU air samples and sputum samples of Western ICU patients in the third quarter of 2022

Paragraph: use this for the first paragraph in a section, or to continue after an extract. We collected sputum samples (specimen number W5) from a long-term hospitalized patient in the western ICU, and compared the pathogens with the western ICU air samples. The results are shown in Figure 5. The pathogens in the sputum of the patient were mainly *A. baumannii*, *P. aeruginosa*, *Streptococcus oralis*, *C. striatum*, *E. faecium*, etc. The main pathogens in the western ICU air samples were *C. tuberculostearicum*, *A. baumannii*, *C. acnes*, *P. aeruginosa*, *M. luteus*, etc. We collected *E. faecium* in ICU air samples and patient sputum samples. Compared with the hospital pathogen distribution table (Table 1) in the quarter, there were two patients with *E. faecium* infection in the Western ICU.

3.5. Summary of the top three pathogens and drug sensitivity of hospital infection cases in the third quarter of 2022

In the third quarter of 2022, a total of 356 strains of pathogenic bacteria were detected in nosocomial infections in the whole hospital, and the top three detected pathogens were *A. baumannii*, *K. pneumoniae* subspecies, and *P. aeruginosa*. Among them, 78 strains of *A. baumannii* were detected (75 strains in sputum and 3 strains in blood), 71 strains of *K. pneumoniae* subspecies were detected (51 strains in sputum, 17 strains in blood, 2 strains in ascites, and 1 strain in secretion), and 50 strains of *P. aeruginosa* were detected (43 strains in sputum, 3 strains in ascites, 2 strains in blood, 1 strain each in secretion and urine). The specific drug sensitivity results are shown in Table 2.
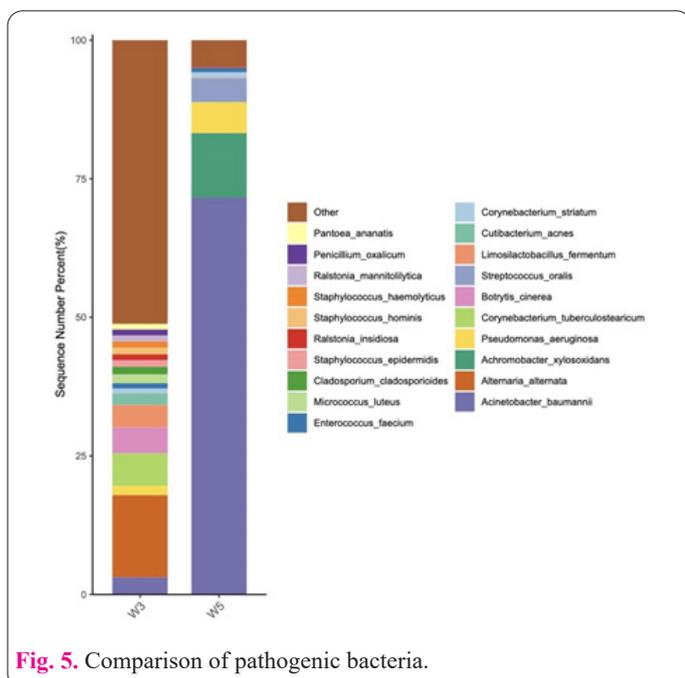

Fig. 5. Comparison of pathogenic bacteria.

The resistance rate of *A. baumannii* to β-lactam antibiotics was higher, and the resistance rate of *P. aeruginosa* to tetracycline antibiotics and second- and third-generation cephalosporins was up to 100%.

4. Discussion

According to the results of the analysis, we did not find a significant difference in microorganisms among the infusion room, general ward and intensive care unit, and there was no significant difference in pathogenic microorganisms between the east and west areas. This indicates that there is no difference in pathogenic microorganisms between different departments and floors, and the distribution of pathogenic microorganisms in different hospital districts is roughly similar. The sampling site selected for the collection of pathogenic microorganisms is a large tertiary hospital in the eastern region. Next, we can select secondary hospitals and primary hospitals for air sample collection to analyze whether pathogenic microorganisms have mobility in different levels of hospitals. We should identify this potential risk of pathogen transmission as much as possible, cut off the transmission route in time, and avoid the flow of pathogenic microorganisms in different levels of hospitals.

We collected *E. faecium* in both ICU air samples and patient sputum samples. Comparing the distribution of pathogens in the hospital during the quarter, we also found two patients with *E. faecium* infection in the western ICU. Therefore, when a patient has a clear pathogen infection, we need to carry out environmental disinfection, including not only terminal disinfection of bed units but also cleaning and disinfection of air conditioning outlets and fresh air systems.

When an infectious patient or a suspected infectious patient that may spread through the central air conditioning ventilation system is found in the hospital, the operating operator should immediately close all the air outlets in the area or immediately stop the central air conditioning terminal equipment in the area. During the use of the hospital central air conditioning system, the air treatment equipment and its inner wall surface should be tested for biological pollutant pollution every 2 months. The fresh air inlet filter should be inspected weekly. When the mesh is blocked more than 50 %, it should be cleaned and disinfected. In addition, the treatment of sewage, sludge and waste gas in medical institutions should also meet the discharge standards of water pollutants in medical institutions. The pathogenic bacteria in sewage should be detected regularly. The monitoring of the atmosphere should be monitored quarterly, and the sampling frequency should be sampled every 2 hours. A total of 4 times were collected, and the maximum measured value was taken; the sludge sampling monitoring adopts multi-point sampling, the sample should be representative, the sample weight should not be less than 1 kg, and the monitoring should be carried out before clearing.

Through traditional culture methods, we believe that the lower respiratory tract of healthy people was sterile. However, it has been shown that the bronchial tree of healthy people is not sterile, with the development of 16S-rRNA gene sequencing [21]. Healthy lower respiratory tract high abundance flora at the phylum level are mostly Firmicutes, Bacteroidertes, Proteobacteria, Fusobacteria, and Actinobacteria, and at the genus level are Streptococ-

Table 1. Pathogen distribution for the third quarter of 2022.

Department	Number of strains	Acinetobacter baumannii		Pseudomonas aeruginosa		Enterococcus faecium		Enterococcus faecalis		Burkholderia cepacia		Proteus mirabilis		Candida albicans		Stenotrophomonas maltophilia		Others	
		N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
Western ICU	27	10	37.04%	6	22.22%	2	7.41%	2	0.0741	2	7.41%	0	0	0	0	0	0	5	18.52%

Table 2. The result of drug sensitivity.

Antibacterial drug	Acinetobacter baumannii (N=78)				Klebsiella pneumoniae subspecies (N=71)				Pseudomonas aeruginosa (N=50)			
	Number of sensitivity	Number of resistance	Susceptibility (%)	Resistance rate (%)	Number of sensitivity	Number of resistance	Susceptibility (%)	Resistance rate (%)	Number of sensitivity	Number of resistance	Susceptibility (%)	Resistance rate (%)
Piperacillin	9	65	12.33	87.67	1	60	1.72	98.27	23	27	46	54
Piperacillin Sodium; Tazobactam Sodium	8	64	10.96	89.04	25	33	43.10	56.90	25	25	50	50
Ticarcillin	7	51	12.07	88.23	0	45	0	100	6	36	14.29	85.71
Ampicillin	0	15	0	100	0	13	0	100	0	8	0	100
Ampicillinand Subactam	2	13	13.33	86.66	3	10	23.08	76.92	0	8	0	100
Amoxicillin and clavulanate potassium	0	73	0	100	27	31	46.55	53.44	0	50	0	100
Ceftazidime	11	62	15.07	84.93	30	28	51.72	48.28	30	20	60	40
Cefepime	12	61	16.44	83.56	24	34	41.38	58.62	32	18	64	36
Cefuroxime	0	68	0	100	21	24	46.67	53.33	0	42	0	100
Cefoperazone sulbactam	9	49	15.52	84.48	28	17	62.22	37.78	19	23	45.24	54.76
Cefazolin	0	15	0	100	3	10	23.08	76.92	0	8	0	100
Ceftizoxime	10	48	17.24	82.76	28	17	62.22	37.78	0	42	0	100
Cefotaxime	3	70	4.11	95.89	30	28	51.72	48.28	0	50	0	100
cefpodoxime	0	58	0	100	24	21	53.33	46.67	0	42	0	100
Cefotetan	0	58	0	100	25	20	55.56	44.44	0	42	0	100
Aztreonam	0	13	0	100	27	31	46.55	53.45	1	1	12.5	87.5
Imipenem	11	62	15.07	84.93	31	27	53.45	46.55	38	12	76	24
Doripenem	7	51	12.07	87.93	28	17	62.22	37.78	31	11	73.81	26.19
Meropenem	11	62	15.07	84.93	31	27	53.45	46.55	39	11	78	2
Tobramycin	24	34	41.38	58.62	27	18	60	40	42	0	100	0
Gentamicin	4	11	26.67	73.33	3	10	23.08	76.92	8	0	100	0
Amikacin	7	8	46.67	53.33	48	10	82.76	17.24	50	0	100	0
Chloramphenicol	0	15	0	100	11	2	84.62	15.28	8	0	0	100
Tetracycline	23	50	31.51	68.49	27	31	46.55	53.45	0	50	0	100
Doxycycline	19	39	32.76	67.24	21	24	46.67	53.33	0	42	0	100
Tigecycline	19	39	32.76	67.25	28	17	62.22	37.78	0	42	0	100
Minocycline	37	21	63.79	36.21	27	21	56.25	43.75	-	-	-	-
Levofloxacin	13	60	17.81	82.19	3	31	46.55	53.45	28	22	56	44
Ciprofloxacin	13	60	17.81	82.19	27	31	46.55	53.45	34	16	68	32
Moxifloxacin	9	53	14.52	85.48	30	28	51.72	48.28	25	17	59.52	40.47
Norfloxacin	9	49	15.52	84.48	24	21	53.33	46.67	39	3	92.86	7.14
Compound sulfamethoxazole	42	31	57.53	42.47	45	13	77.59	22.41	0	50	0	100
Polymyxin B	0	15	0	100	0	13	0	100	0	8	0	100
Colistin	0	58	0	100	0	45	0	100	0	42	0	100
Cefoxitin	-	-	-	-	24	21	53.33	46.67	-	-	-	-
Doxycycline	-	-	-	-	3	0	100	0	-	-	-	-
Ticarcillin-Clavulanate	-	-	-	-	-	-	-	-	14	28	33.33	66.67

Monitoring and analysis of pathogenic

Cell. Mol. Biol. 2024, 70(4): 181-190

cus, Staphylococcus, Veillonella, Prevotella, and Hemophileae [22–24]. There are colonized microorganisms in the lower respiratory tract of the human body. These microbial communities constitute the lower respiratory tract microbial homeostasis. When the human body is in a state of low immunity or the homeostasis environment is broken, the respiratory tract flora will change.

The advent of metagenomics next-generation sequencing (NGS) has filled the gap in the discovery of pathogenic microorganisms by traditional microbial culture and allows the detection of all known and unknown pathogens. NGS has been widely used in clinical practice to analyse the microbial composition of patients' lower respiratory tract. The use of NGS allows for rapid diagnosis, precise treatment, and improved patient prognosis. However, the results of NGS have a certain degree of false positives, and there is a possibility of contamination during both sample collection and sequencing. Therefore, in clinical practice, we need to combine the patient's case, biochemical indicators, and imaging features to determine the final diagnosis of the patient.

The main resistance genes (except catI) in each sampling site can be found in other sampling sites, and the microbial resistance genes were not significantly different among the three groups in outpatient, inpatient and intensive care units. However, there were three resistance genes in the eastern region and the western region, which indicates that there may be resistance gene migration. According to research, air is also an important route for the spread of resistance genes, especially by inhaling fine particulate matter in the air (PM2.5) [25]. Horizontal gene transfer (HGT) is considered to be the main route of antibiotic resistance gene transmission, but vertical gene transfer (VGT) is also involved in its transmission [26]. The use of antibiotics can lead to the production of antibiotic-resistance genes, which can be transmitted from the environment to humans through water, food, skin contact and inhalation [27]. Bacteria can evolve into new drug-resistant strains through gene mutation or horizontal transfer of ARGs. The long-term use of antibiotics will cause horizontal transfer of antibiotic resistance genes. If bacteria that can reproduce in the human body are resistant to a variety of antibiotics and can produce virulence, new superbacteria will be formed. Therefore, we must control the use of antibiotics, not excessive, abuse of antibiotics. Since the end of 2019, China has implemented centralized procurement nationwide. Some broad-spectrum antibiotics that have entered centralized procurement have low prices and good anti-infective effects, but our choice of antibiotics cannot be completely determined by the price, and we should take into account the grade of antibiotics.

Compared with the results of 2016 [28], the types of pathogenic microorganisms in the hospital environment had changed. At that time, the dominant bacteria in the hospital environment were *Staphylococcus saprophyticus*, Corynebacterium, *Streptococcus pneumoniae*, *E. coli* and *P. aeruginosa*, and the main pathogenic bacteria were *S. pneumoniae*, *E. coli* and *P. aeruginosa*. Therefore, with the migration of time, the composition of pathogenic bacteria in hospitals is constantly changing. We should regularly monitor the changes of pathogenic microorganisms in the air. When new pathogenic microorganisms are found, intervention can be carried out as early as possible to identify the risk of potential pathogen transmission, timely cut off the transmission route, and avoid the flow of pathogenic microorganisms in different levels of hospitals.

Pathogenic microorganisms in the air are an important cause of respiratory diseases, which can act directly on the immune system and exert immune effects by affecting the respiratory commensal flora [29]. Studies have shown that the number of opportunistic pathogens increased significantly during the development of respiratory diseases (such as chronic obstructive pulmonary disease) [30]. We had a high degree of overlap in the composition of pathogenic bacteria in the patient's sputum and in the air samples from the ward (West ICU) where they were located and considered the following possible causes. Patients in a deep coma after craniocerebral haemorrhage or severe craniocerebral injury craniotomy, lost normal cough reflex and swallowing function, so that respiratory secretions cannot be eliminated autonomously, and vomit or regurgitated material can easily reflux into the respiratory tract, and long-term use of ventilator-assisted breathing, tracheal intubation makes the original relatively sterile lower respiratory tract directly exposed to the outside while increasing the difficulty of oral cleaning. The oropharyngeal colonizing bacteria proliferate, and the oropharyngeal secretions containing a large number of colonizing bacteria enter the lower respiratory tract through the gap between the air sac and the wall of the air tube under the action of various factors. The muscle strength of the upper cardia sphincter in patients after cardiac vascular surgery is weak, and the nasogastric tube is often retained after cardiac vascular surgery, which can lead to gastric retention and reflux in patients so that the gastric contents are inhaled into the trachea to block the bronchus and form atelectasis. It can also cause reflux of gastric contents and aspiration to the lower respiratory tract, resulting in bacterial translocation, thus leading to lung infection. At the same time, the existence of tracheal intubation makes the patient unable to cough effectively, interferes with the clearance function of cilia, and reduces airway protection ability. The use of analgesic and sedative drugs can inhibit the cough ability of patients, thus increasing the risk of ventilator-associated pneumonia (VAP). Long duration of postoperative tracheal intubation is prone to ventilator dependence, which not only destroys the natural defense barrier of the airway and brings external bacteria into the patient's body, but also leads to damage of respiratory mucosa, inability to timely discharge lung secretions and poor ability to remove foreign invading pathogens. Meanwhile, long-term catheter retention in the patient's body makes it easier for pathogens to attach and multiply, increasing the risk of lung infection.

5. Conclusion

Among the pathogenic bacteria found in the hospital, *A. baumannii* was detected in all sampling points in our hospital. Unlike traditional ideas, the relative abundance of *A. baumannii* was not the greatest in the ICU, but in the outpatient transfusion room. No significantly different microorganisms were found in the infusion room, general ward and intensive care unit. Therefore, it is considered that there is *A. baumannii* infection in the community, and it is necessary to strictly control the hierarchical management of antibiotics in the community and pharmacy. The next step is to detect the airborne pathogenic microorganisms and resistance genes in the secondary hospitals and

Monitoring and analysis of pathogenic

Cell. Mol. Biol. 2024, 70(4): 181-190

community health service centers to understand the epidemiological distribution.

The resistance genes in tertiary hospital were mainly related to macrolide antibiotics and aminoglycoside antibiotics, which can provide reference for outpatients and inpatients in the selection of antibiotics. The detection of drug-resistance genes does not necessarily mean that the associated pathogens are carried, nor does it mean that the associated pathogens will be resistant.

Compared with the routine body fluid test of pathogenic microorganisms: at the same time node of air sample collection in the hospital, the pathogenic microorganism analysis report issued by the hospital infection disease management office mainly included *A. baumannii*, *S. aureus* and *P. aeruginosa*, which were similar to the composition of pathogenic microorganisms in the air medium of the sample hospital. Therefore, the detection of pathogenic microorganisms and resistance genes in the air is more sensitive than conventional detection. After regular detection of pathogenic microorganisms and resistance genes in the air, the target pathogenic microorganisms were immediately subjected to hospital infection prevention and control measures such as air purification and disinfection.

At present, nosocomial infections are increasing year by year, and multidrug-resistant bacteria are constantly mutating in different times and spaces, so it is necessary to monitor multidrug-resistant bacteria. Therefore, the detection of resistance genes of drug-resistant bacteria in aerosols between different departments in the hospital and the judgment of whether the drug-resistant bacteria are homologous can evaluate the harmfulness of microbial aerosols in the hospital environment and the transmission route of drug-resistant bacteria in the air, to control the outbreak of nosocomial infection and formulate a plan to control air transmission. At the same time, according to the detection of resistance genes, we can understand the variability of resistance genes of drug-resistant bacteria, adjust antibiotics in time, and determine the medication plan.

Informed Consent
The authors report no conflict of interest.

Availability of data and material
We declared that we embedded all data in the manuscript.

Authors' contributions
XK, DX and YM conducted the experiments and wrote the paper; CB, ZL and DC analyzed and organized the data; HT conceived, designed the study and revised the manuscript.

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