DISTRIBUTION AND GENE DETECTION OF DRUG-RESISTANT BACTERIA IN THE AIR OF HOSPITAL

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ABSTRACT

Objective: At the environmental level, we should monitor the resistance genes in the air to control the occurrence of nosocomial infections. 2. The distribution of pathogenic microbes in different sections and seasons is different, and the resistance genes of resistant bacteria in different sections and seasons are detected to know whether the resistance genes in each department are homologous, so as to monitor the role of resistant bacteria in the air. 3. Detection of antagonistic genes can be used to understand the resistance of resistant bacteria to antibiotics, to discover the variation of resistance genes in time, and to guide the drug use scheme of drug resistant bacteria in this area.

Methods: 1. Hospital air sample collection: 10 sampling points including the hospital corridor, emergency infusion, general outpatient, Department of respiration ward, neonatal ward, general paediatric ward, digestive ward, ICU ward, Department of cerebral surgery ward, urology ward, were used as the air medium sampling point, and the investigation of resistance gene pollution were conducted in four seasons: spring, summer, autumn and winter. 2. Qualitative and quantitative analysis of resistance genes: the target samples were collected for qualitative and quantitative analysis of target genes in the laboratory.

Results: 1. All the air samples in the hospital environment were integrated to analyze the proportion and structure of the pathogenic microorganism in the hospital environment. The proportion of the number of pathogenic microbes in the hospital environment accounted for 3.64% of the total number of all the analytical samples. 2. In different seasons, the main pathogenic bacteria are different, and the drug-resistant bacteria are also different. In the general ward, ICU has different resistance genes. 3. The content of macrolide resistance gene was significantly higher than that of beta lactam and quinolone.

Conclusions: (1) the basic status of resistance gene pollution in our hospital: The distribution of resistant bacteria in the air of general departments of hospitals and their resistance genes are homologous, because of the change of the first five drug-resistant bacteria with the seasonal changes, so we need to improve the daily disinfection measures of air conditioning and ventilation equipment and cut off the transmission routes every quarter. It is more effective to control the sense of the hospital. (2) The comparison of resistance genes of resistance bacteria in various quarters of the hospital to the conventional culture specimens avoids the repeated detection of the same patient, and is more sensitive to the difficult culture bacteria than the conventional culture specimen. (3) Establish a resistance gene library of some drug-resistant bacteria in our hospital. In each quarter, each hospital department of pathology issued a microbiological room for different departments of drug resistant bacteria statistics. To increase the monitoring of drug resistance genes, improve the sensitivity of the original drug resistant bacteria monitoring, and reduce the biological pollution in the hospital environment can cause a series of epidemic outbreak of infectious diseases.

Keywords: Resistance genes, drug-resistant bacteria, hospital air monitor.

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Background

Nosocomial infection has become a major concern in the world today\(^1\). Its refractory and high incidence and mortality have become a new subject in clinical practice\(^2\). Hospital infection not only prolonged the hospitalization period, but also increased the fatality rate. It brought great threat to the life and health of patients, and the economic loss was serious\(^3\).

The nosocomial infection rate of 16 hospitals in China from November 1987 to October 1988 showed that the incidence rate was 9.7%. If according to the statistics of the Ministry of health in 1989, there are about 50 million hospitalized patients nationwide, of which about 5 million cases of hospital infection have occurred, and all kinds of losses are considerable. The serious disease often causes the patient's disease to fail to achieve the expected curative effect and even cause death.
In the nosocomial infection, the main infection rate was 1-2 of drug-resistant bacteria. Multidrug-resistant bacteria, closely related to nosocomial infection, have become the three leading infectious diseases in the world with HIV and hepatitis. In recent years, pathogen variation and antimicrobial drug abuse have become a new topic of nosocomial infection management and clinical diagnosis and treatment. To establish and improve the monitoring in infection management and clinical diagnosis and drug abuse have become a new topic of nosocomial infection, have become the three leading infectious diseases in the world with HIV and hepatitis. In recent years, pathogen variation and antimicrobial drug abuse have become a new topic of nosocomial infection management and clinical diagnosis and treatment. To establish and improve the monitoring in infection management and clinical diagnosis and drug abuse have become a new topic of nosocomial infection management and clinical diagnosis and treatment.

To control the spread of multidrug-resistant bacteria, we need to understand the variety and distribution of multidrug-resistant bacteria, to strengthen the education and training of the staff, to clean the environment and articles correctly, and to strictly isolate the infected patients with multidrug-resistant bacteria. This is an effective way to prevent and control the spread of multidrug-resistant bacteria.

At present, there are few studies on air microbes and ARGs pollution in urban hospitals, and lack of basic data on the exposure risk of crowd in hospital environment. The hospital is a special place in the city. There are a wide variety of pathogens in the hospital environment. The pathogen may invade the human body through various environmental media, especially the patients of the susceptible population, thus causing hospital infection. Air, as a major communication medium, accounts for the first of all infectious routes. The number and variety of the air microbes in the hospital are more complex than the general environment, and the pathogenic microbes in the hospital may bring the risk of infection to the population by means of respiratory tract, such as the respiratory tract. In addition, the hospital is one of the main places directly related to antibiotics and the human body. The level of microbial pollution in the hospital environment is studied by culture method, so as to optimize the sampling conditions. The results show that the environmental air microorganism pollution in the hospital belongs to the level of clean standard. The sampling method recommends a large flow sampler to sample 20~30 h in order to meet the analysis of the follow-up experiment. The optimum pretreatment conditions were 52 cm² sampling film, 25 mg magnetic beads and 40 ml buffer solution. Qubit fluorescence method was used for quantitative determination of DNA samples in hospital environment. The obtained DNA samples can meet the requirements of subsequent high-throughput sequencing technology and real-time fluorescence quantitative polymerase chain reaction (qPCR).

At present, a variety of multidrug resistant bacteria have been detected in the hospital environment, and some of them belong to the pathogenic bacteria, such as methicillin resistant Staphylococcus aureus and vancomycin resistant Enterococcus, which can survive in the environment for a long time and have the risk of disease. At the same time, ARGs in the resistant strain or exposed environment can spread and spread in the environment through gene level transfer, increasing the risk of ARGs pollution. The presence of ARGs in the hospital ambient air or pathogenic bacteria carrying ARGs can cause infection through the respiratory tract and pose a threat to human health.

Some pathogenic bacteria are at risk of infection. Studies have shown that a very small number of pathogens in the air can cause respiratory infections in humans and animals. The microbial aerosol in the range of 4~20 μm can cause human disease, and the smaller the particle size, the deeper the body is, the greater the harm to the human body. If the particle size in the range of 4~5 μm can reach the bronchial mucosa, most of the microorganism particles in the range of 1~5 μm can reach the alveoli and cause the respiratory infection of the human body.

Materials and methods

Experimental Methods

Sample collection and preprocessing method

Collection of airborne microorganism samples in hospital environment

• Method of collecting air microorganism

Microbes exist in the form of aerosols in the air, and the size range of the microbial aerosol is 0.002 to 30 μm, and the size of the disease related to human disease is 4~20 μm. Air microorganism collection is the process of enriching airborne microbes in the sampling medium (solid, semi-solid or liquid) or sampling membrane by some methods. According to the sampling principles of airborne microorganisms, the sampling methods of airborne microorganisms can be divided into two main categories: natural sedimentation method and airflow impact method.

• Natural sedimentation method

It is a method of enriching the microorganisms in the sampling area on the surface of the medium in a certain period of time by using the gravity effect of microorganism aerosol particles. As a classical sampling method for air microorganisms, the natural sedimentation method has the advantages of simple operation and economy, and has been widely applied at home and abroad. For example, Gong Qing Yue et al. has used natural sedimentation to monitor the air bacteria in clean operation room.
However, the sampling of natural sedimentation is related to the size of aerosol particles, and the particles with smaller particle size are not easy to settle, which makes the sampling results lack accuracy in qualitative and quantitative, and is easily affected by the sampling conditions of wind speed and wind direction\(^9\).

**Airflow impact method**

The airflow impact method is to use the sampling device to draw the airstream onto the surface of the sampling medium so as to enrich the microorganisms in the airstream.

**Collection of total air microorganism samples in hospital environment**

A large flow TSP sampler was used to collect the total air microorganism samples of the hospital environment. The sample film was made of glass fiber filter membrane, and the sampling rate was set from 0.5 to 1 m\(^3\) according to the different sampling environment.

**Sampling point and quantity**

- 10 sampling points such as hospital corridor, emergency transfusion, general outpatient, Department of respiration ward, neonatal ward, general paediatric ward, digestive ward, ICU ward, Department of cerebral surgery ward, urology ward, were used as sampling points of air medium. Investigation of four-time resistance gene contamination was conducted in spring, summer, autumn and winter.

The number of sampling points is determined according to the size of the monitoring room and the scene. In principle, a room less than 50 m\(^2\) should have 1 to 3 points, 50 to 10 m\(^2\) for 3–5 points, and 100 m\(^2\) for at least 5 points. Distributed evenly on a diagonal line or in a plum blossom type. At the same time, it is sampled at the outlet of the air conditioner and the outlet of the disinfecter. After the qualitative and quantitative analysis of resistance genes, the contents of resistance genes in air environment and the distribution characteristics of resistance genes in different microorganisms were studied.

**Qualitative and quantitative analysis of resistance genes**

The target samples were collected for qualitative and quantitative analysis of target resistance genes. It includes sample collection, preservation and preprocessing, extraction of nucleic acid in environmental samples, construction of biological standard, qualitative and quantitative PCR method, and whole process quality monitoring. This link is the innovation link of this research, making trace.

The extracted DNA was used as a template. The qualitative and quantitative detection of resistance genes were characterized by means of common PCR, RTQ-PCR, flow cytometry and trace nucleic acid quantitometry (Nanodrop), etc.

The study of resistance genes in air environment focuses on indoor air environment in hospitals. The content of resistance genes in air environment and the distribution characteristics of resistance genes in different microorganisms were studied. Unlike other media, the resistance gene contamination in the atmosphere directly enters the human body through inhalable fine particles, which has a significant impact on human health. Therefore, it is very important to study the resistance genes in the atmosphere, and to study the resistance genes directly related to human health, especially the pathogens. Therefore, in addition to studying the types and contents of resistant genes in the atmosphere, we focused on the resistance genes carried by microorganisms.

**Results**

**Study on bacterial aerosol**

**Bacterial aerosol concentration**

In order to study the condition of environmental air bacteria pollution in hospital, the hospital environment was sampled with the Anderson six level impact sampler, and the sampling points were designed in three departments of the hospital infusion area, outpatient hall and inpatient department, and the clean medical environment (the new Department of Pediatrics of the hospital) was used as the control.
The concentration of airborne bacteria in different departments of the hospital is shown in Figure 1.

The air bacteria concentration at all sampling points is within the range of 102~105 CFU/m³. The concentration of air bacteria in the three departments of the hospital environment transfusion area, outpatient department and inpatient department are 636 ± 245, 884 ± 500 and 278 ± 28 CFU/m³ respectively. It is close to the clean medical environment and the general indoor environment, and the difference is two with the crowded area. Grade. According to the air microorganism evaluation standard set by the ecological environment research center of the Chinese Academy of Sciences, the environmental air of the hospital is a clean standard (<1000 CFU/m³; the study shows that the concentration of air bacteria in the outpatient Hall of the hospital is up to 1281 CFU/m³, slightly higher than the results of this study.

Li and Hou (2003) studied the environment of 100000 clean wards, and found that the bacterial concentration in the hospital ward environment was within the range of 1~423 CFU/m³, and the results of this study were in accordance with the results. In addition, studies have shown that human flow has a greater impact on air microbes, and there is a positive correlation between air microbial concentration and human flow rate. In the three departments of the hospital environment, the air bacteria concentration outpatient department, the infusion area and the inpatient department may be because the human flow in the outpatient hall is the largest, and the human hair and debris are easily formed by the microbial aerosol in the environment.

**Analysis of pathogenic microorganism in air**

The proportion and structure of pathogenic microorganisms in the hospital air environment were analyzed by the integration of all air samples from the hospital environment. The results are shown in Figure 4-10. According to the analysis, the proportion of the number of pathogenic microbes in the hospital environment accounted for 3.64% of the total number of the total analysis samples, which indicates that the pollution level of the environmental pathogenic bacteria in the hospital is still more severe. In addition, the leading bacteria in the hospital environment were staphylococcus saprophyticus (30.74%), Corynebacterium (10.99%), Streptococcus pneumoniae (10.42%), Escherichia coli (11.26%), and Pseudomonas aeruginosa (9.48%). However, most of the pathogens were Streptococcus pneumoniae, Escherichia coli and Pseudomonas aeruginosa.

**Figure 2:** Analysis of airborne pathogenic microorganism.

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**Figure 3:** Differences in pathogen composition between typical hospitals in summer and winter.

**Figure 4:** The relative concentration of ARGs in different ambient air in hospitals.

**Detailed investigation of pathogenic microorganism in typical hospital**

**Seasonal variation of airborne pathogenic microorganisms in Typical Hospitals**

In order to study the change of the pathogenic microorganism of the hospital environment in different seasons, the hospital air samples collected in
summer and winter in our hospital were analyzed, and the results were compared. The proportion of pathogenic microorganisms showed little difference between summer and winter, and winter was slightly lower than that of summer. The proportion of the pathogenic microbes was significantly different between the two seasons. In summer, the proportion of Escherichia coli and Pseudomonas aeruginosa was significantly higher than that in winter, while the proportion of Enterobacter aerogenes and Streptococcus pneumoniae was higher in winter.

Study on the characteristics of ARGs pollution in hospital environment

The qPCR technology was used to quantitatively study the ARGs pollution in the hospital environment, revealing the characteristics of ARGs pollution in the hospital environment and its differences in the regional, hospital departments and seasons. According to the potential dose values of bacterial aerosol, pathogens and ARGs in the hospital environment, the exposure risk assessment of hospital environmental air biological pollutants was carried out.

In order to study the pollution status of ARGs in the environmental air medium of the hospital, all the air samples in the hospital environment were counted, and the different kinds of ARGs concentration were compared and analyzed. The results were shown as shown. The 12 ARGs genes of beta lactam blaTEM and macrolide ermB genes are the two highest relative concentrations, 6.25x10^7 copies/ng DNA and 2.46x10^6 copies/ng DNA respectively. blaTEM and ermB genes are the genetic types of higher concentrations in the hospital environment.

Analysis of ARGs quantitative results in different sectors of air medium

All hospital air samples were divided into different areas, namely, transfusion area, outpatient department and inpatient department. The pollution characteristics of three categories of resistance genes among different departments were analyzed and quantitatively analyzed. It can be seen from the graph that the relative concentration of the three major resistance genes in three sectors is consistent, which is consistent with the law in section 5.2.1.2. However, the size and size of different kinds of resistance genes were not consistent, among which beta lactam was most distinct between the three departments, infusion area > outpatient department > inpatient department, and the other two in three departments. According to the comparison between departments, the beta lactam and quinolones ARGs in the infusion area are higher than those of the other two departments. The analysis of the reasons may be related to the environment of the infusion area. There are more patients in the infusion area, and the continuous admission of patients during the day, the intensive flow of people and the activities of the incoming people lead to the exogenous input of the unknown ARGs, which may be one of the reasons for the serious contamination of the resistance genes in the infusion area.

Comparison of seasonal distribution of four kinds of ARGs in typical hospital environment

The study selected these four kinds of ARGs: blaTEM, blaCTX-M, mecA, ermB as typical ARGs species. blaTEM and ermB belong to the high concentrations of genes in the hospital environment. The blaCTX-M gene is often associated with multiple drug resistance, and the mecA gene is the resistance gene of methicillin resistant Staphylococcus aureus (MRSA). It is of great significance to study the distribution characteristics of these typical ARGs in the hospital environment. The pollution status of typical ARGs in winter and summer two seasons in the hospital air environment was compared and analyzed.
The relative concentration results of the hospital environment air medium in winter and the typical ARGs in summer were shown in Figure 6. In the two seasons of winter and summer, the relative concentrations of four typical ARGs were the same, that is, blaTEM > ermB > mecA > blaCTX-M. In the four seasons, the relative concentrations of the four ARGs were not very different, and the concentrations of blaTEM and mecA genes in winter were slightly higher than those in summer.

Comparison of laboratory results of drug-resistant bacteria in 2016

In 2016, 492 strains of pathogenic bacteria were detected in the hospital infection cases, and the top five pathogens were identified as: Western ICU (92 strains), Western Department of thoracic surgery (57 strains), East Pu external gastrointestinal (35 strains), Eastern surgery ICU (30), and West Department of orthopedics (11). The first five pathogens in hospital infection were Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, and Acinetobacter Bauman. There were 80 strains of Escherichia coli (17 in sputum, urine, 15 in the blood, 11 in the secretions, 9 in the ascites, 7 in the pus, 2 in the bile, 1 in the nasopharynx swab, and 1 in the drainage), and 67 Klebsiella pneumoniae (50 in sputum, 8 in the blood, 4 in pus, 3 in ascites, and secretory. There were 55 strains of Staphylococcus aureus (19 strains, 18 sputum, 12 in sputum, 3 in pus, 3 in blood, 1 in urine, chest water and ascites), and 52 strains of Pseudomonas aeruginosa (35 in sputum, 8 in secretions, 5 in pus, blood, urine, ascites and nasopharynx) in 1 strains of Staphylococcus aureus. Among the 1 swabs, 44 strains of Acinetobacter baumannii were found in Bauman (39 strains in sputum, 3 in secretions, 1 in pleural effusion and ascites). Specific drug sensitivity was shown in Table 1.

The main pathogenic bacteria in the air are Streptococcus pneumoniae, Escherichia coli and Pseudomonas aeruginosa. The pathogenic bacteria in the human specimens of ward disease found that the first five pathogens were Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa and Acinetobacter Bauman. But as pathogenic bacteria, we still think that Streptococcus pneumoniae, Escherichia coli and Pseudomonas aeruginosa in the air are roughly similar in the air, and the proportion of Klebsiella pneumoniae is higher than that in the front. But for hospital statistics, because of the use of antibiotics and repeated sampling of the same patient, there is repeatability and false negative rate.

Discussions

• At present, the detection of resistance genes in the air, with the development of molecular biology technology, non culture based methods such as denaturation gradient gel, restriction fragment length polymorphism analysis, quantitative PCR, microarray, etc. are also widely applied to the study of air microorganism. In addition, the innovation and development of the two generation sequencing.
technology are also microbiology. A new field has been opened up in the study. It is possible for us to get microbiological coding in the air. Based on the high false negative rate of traditional human specimen culture, the detection of resistance genes in air is superior to traditional culture.

• There are few studies on ARGs in the air environment of hospitals at home and abroad. Most of them are concerned about MRSA related mecA genes, and lack of research on other ARGs systems. Most of the research methods are based on the cultivation of ARGs in resistant strains, and lack of research on ARGs of all microorganisms in the environment. Whether MASA is a drug-resistant gene carried by nosocomial infection or community acquired infection, we need to compare the MASA gene in the community.

• The detection of resistance genes in air was consistent with the surveillance of drug resistance in infectious diseases department of our hospital. In the detection of resistant genes in air, we can see that due to different ventilation systems, the concentration of drug-resistant bacteria is different, but the proportion of drug-resistant bacteria is similar. Special departments, such as ICU, are different from the general ward.

• The future research prospects: the detection of drug resistant genes in hospital infection in this study, and the results have a great guiding role in the monitoring of nosocomial infection. For the present situation of community acquired infection, the common culture medium of common pathogens is difficult to be cultivated first. The positive rate of specimen culture is about 15% every year, and most of them are nosocomial resistant bacteria. Most of them are repeated culture. Because of the easy access to community antibiotics and the extensive use of antibiotics in animal husbandry and aquaculture, these resistant genes can be transmitted through air, soil, etc., even with human pathogens, but the high false negative rate of culture brings difficulties to the clinical work, because of the pathogens in the community, because of the pathogens. The two generation sequencing of pathogens is currently difficult to determine, but the cost is high. In large hospitals and other large hospitals in Shanghai, the two generation of the respiratory tract pathogens are often sequenced because of the high drug resistance of the macrolides, which can be used for the use of macrolides, but for individuals, the two generation sequencing is costly and has a long cycle. Therefore, for the community acquired infection, the surveil-

lance of the pathogenic bacteria in the region and the sequencing of the resistant genes can make the pathogen clear as soon as possible, improve the common pathogens in the region, monitor every quarter and guide the treatment in the next quarter. How to reduce the cost. For community-acquired infections, the resistance genes in the air can be tested according to local drug sensitivity and bacterial spectrum. For the study of translational medicine, how to apply bioengineering to clinic needs further cooperation and extensive use. Next, we need to detect more gene sequences and compare the resistance genes. The discovery of a new drug resistant gene.

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