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RESEARCH ARTICLE

Application of metagenomic next-generation sequencing in pathogen detection for patients with lower respiratory tract infections caused by multidrug-resistant organisms and analysis of related factors

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ABSTRACT

The incidence of lower respiratory tract infections (LRTIs) caused by multidrug-resistant organisms (MDRO) has been high in recent years. However, traditional etiological detection methods have not been able to meet the needs for clinical diagnosis and prognosis of LRTIs. The rapid development of metagenomic next-generation sequencing (mNGS) provides new insights for diagnosis and treatment of LRTIs. We conducted a retrospective study on 95 patients with lower respiratory tract infections caused by MDRO admitted to our respiratory department from January 2022 to December 2023. These patients underwent mNGS testing and conventional culture testing. Additionally, 150 patients without lower respiratory tract infections caused by MDRO during the same period were included as the non-MDRO group. General information was collected, and Logistic regression analysis was performed to identify risk factors for MDRO infections in patients with lower respiratory tract infections. Our results show that the time to pathogen detection by mNGS was 50.76 ± 1.730 h, that is significantly shorter than 55.53 ± 2.782 h required for conventional culture testing. The pathogen detection rate by mNGS was 89.47% (85/95), higher than the 67.37% (64/95) identified by conventional testing. In terms of pathogen genus distribution, mNGS detected a total of 279 pathogens, while conventional testing detected 121 pathogens. Logistic multivariate regression analysis identified that the use of more than two antibiotics, invasive procedures, invasive mechanical ventilation for ≥7 days, and stay in the respiratory intensive care unit (RICU) for ≥7 days were the main influencing factors for lower respiratory tract infections caused by MDRO ($P < 0.05$).

KEYWORDS

bronchoalveolar lavage fluid, metagenomic next-generation sequencing, lower respiratory tract infection, multidrug-resistant organisms, pathogenic bacteria

INTRODUCTION

The incidence of lower respiratory tract infections has been high in recent years. A large number of broad-spectrum antibiotics are used in clinical treatment. The abuse of antibiotics has also gradually increased, resulting in a gradual increase in antibiotic resistance [1]. The

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incidence of multi-drug resistance, pan-drug resistance, and total drug resistance has increased unabated, seriously threatening the health of patients [2, 3]. Therefore, it is of great significance to explore the risk factors of multi-drug resistant bacteria infection in patients with lower respiratory tract infections. Early and rapid identification of multidrug resistant bacteria infecting the lower respiratory tract, and selection of effective antibiotic treatment is the key to improve prognosis. At present, routine microbial culture is still the gold standard for identifying pathogens, but with low positive rate, long detection time and potential to delay treatment easily [4]. Metagenomic next generation sequencing (mNGS), which obtains genetic information of pathogenic microorganisms through high-throughput sequencing of gene fragments in specimens, has a short detection time and high sensitivity, and can identify pathogenic pathogenic microorganisms [5]. It has been widely used in the diagnosis and treatment of infectious diseases. At present, there are few reports of prospective studies on the application of mNGS in the pathogenic diagnosis and treatment of patients with multi-drug-resistant bacterial infections in the lower respiratory tract. This paper retrospectively analyzed the clinical data of patients with lower respiratory tract multidrug-resistant bacterial infection diagnosed and treated in the Department of Respiratory Medicine of our hospital in recent years, detected the pathogens of patients with lower respiratory tract multidrug-resistant bacterial infection by mNGS technology, analyzed the related factors affecting lower respiratory tract multidrug-resistant bacterial infection, and guided clinicians to use antibiotics rationally, aiming to reduce the risk of lower respiratory tract multidrug-resistant bacterial infection.

METHODS

General information

A retrospective analysis of 95 patients with lower respiratory tract multidrug-resistant bacterial infection admitted to the Department of Respiratory Medicine of our hospital from January 2022 to December 2023, who underwent mNGS testing and routine testing and were cultured into a multidrug-resistant bacterial group, including 59 males and 36 females, aged 56–86 years, with an average age of (72.53 ± 7.03) years. 150 patients without lower respiratory tract multidrug-resistant bacterial infection during the same period were included in the non-multidrug-resistant bacterial group, including 85 males and 65 females; aged 58–85 years, with an average age of (72.73 ± 7.31) years. Inclusion criteria in this study: (1) Meet the diagnostic criteria for lower respiratory tract infection in the "Diagnostic Criteria for Hospital Infection (Trial) " of the Ministry of Health of the People's Republic of China [6]. (2) Multiple drug-resistant bacterial infection of the lower respiratory tract was present at the time of admission. (3) There was a record of antibiotic adjustment based on the results of mNGS in the course of the disease. (4) Informed consent of the patient. Exclusion criteria: (1) BALF was sent for mNGS but not for routine testing. (2) Concomitant with extrapulmonary infection. (3) Concomitant with multidrug-resistant bacterial infection of other sites. This study has been approved by the Medical Ethics Committee of our hospital (Ethical approval number is: Medical Ethics Review [2024] IIT No. (52)).

Pathogen detection

① mNGS detection: For patients with lower respiratory tract infections caused by multi-drug resistant bacteria, at least 5 mL of bronchoalveolar lavage fluid (BALF) should be collected under a flexible bronchoscope and placed in a sterile sputum tube. Immediately after specimen collection, all specimens should be refrigerated at 4 °C and sent to the BGI Genomics Sequencing Center in Guangzhou within 24 h for metagenomic next-generation sequencing (mNGS) detection. Sequencing was performed on PMSEQ4500 platform. The mNGS detection method is as follows: DNA extraction according to the steps described in the instruction manual of the TIANMicrobe magnetic bead-based pathogen microorganism DNA extraction kit (NG550-01). For the extracted nucleic acids, enzymatic digestion for fragmentation, end repair, adapter ligation and PCR reaction to construct a library. Agilent 2100 Bioanalyzer was used to control the quality of the library fragment size, which should be around 300 bp. Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific Inc.) was used to control the concentration of the DNA library. According to the detected concentrations, the constructed libraries were pooled with equal mass. The pooled and mixed libraries were then circularized to form a single-stranded circular structure. Subsequently, through rolling circle amplification (RCA), DNA nanoballs (DNBs) were generated. The prepared DNBs were loaded onto the sequencing chip and sequencing was conducted using the BGISEQ-50/MGISEQ-2000. The data that can be aligned to the human reference genome sequence were removed from the high-quality data through alignment using BWA (BWA: http://bio-bwa.sourceforge.net/). After removing the low-complexity reads from the remaining data, the number of sequences were obtained that can be matched to a certain pathogen based on BGI PMDB pathogen database. Based on the number of sequences and other clinical tests the possible pathogens were determined.

② Routine testing: At least 10 mL of alveolar lavage fluid samples are sent to the microbiology room for microbial culture.

General information

Through the electronic medical record system of inpatients, the relevant clinical data of the selected cases were collected and analyzed, including: basic situation, basic disease, invasive operation, mechanical ventilation, antibiotic application, smoking, admission to the respiratory intensive care unit (Respiratory Intensive Care Unit, RICU), etc., and compared and analyzed the microbial culture and mNGS detection results of pathogen alveolar lavage fluid in

95 patients. This study is a retrospective study, and the sequencing data were not included in the database.

Statistical method

We use SPSS22.0 statistical software for data analytics. Measurement data in line with normal distribution were expressed as ($x \pm s$), and t test was used. Counting data were expressed as rate (%), and Chi-Square tests were used. Univariate and multivariate Logistic regression analysis were performed on various risk factors for multi-drug resistant bacteria infection in the lower respiratory tract, and the difference was statistically significant with $P < 0.05$.

RESULTS

Comparison of detection time and detection rate of pathogens in patients with multidrug-resistant lower respiratory tract infection by mNGS technology and routine detection. The detection time of pathogens detected by mNGS was significantly shorter (55.76 ± 1.730) h than that by conventional detection and culture (55.53 ± 2.782) h, and the difference was statistically significant ($P < 0.01$). 85 cases were positive and 10 cases were negative, and the detection rate was 89.47% (85/95). Conventional detection was positive in 64 cases and negative in 31 cases, and the detection rate was 67.37% (64/95). The difference was statistically significant ($P < 0.05$). See Table 1.

Comparison of pathogenic microorganisms detected by mNGS technology and routine detection in multidrug-resistant bacteria infection of lower respiratory tract. A total of 279 strains of pathogens were detected by mNGS technology, including 200 strains of bacteria, 47 strains of viruses, 26 strains of fungi and 6 strains of special pathogens. Among them, the bacterial infection was mainly *Klebsiella pneumoniae*. Besides, 1 case of Pneumocystis yersini was detected in fungi, and 1 case of Chlamydia parrot was detected in special pathogens. A total of 121 strains of pathogens were detected by routine testing, including 88 strains of bacteria, 20 strains of viruses, 11 strains of fungi and 2 strains of special pathogens, of which the bacterial infection was mainly *K. pneumoniae*; see Table 2.

Univariate analysis of multidrug-resistant infections in the lower respiratory tract. Univariate analysis showed that the main risk factors of MDRO infection in the multidrug-resistant bacteria group were the time of stay in the RICU≥7 days, the use of more than 2 antibiotics, invasive operation, and invasive mechanical ventilation ≥7 days ($P < 0.05$). See Table 3.

Table 2. Comparison of mNGS technology detection and conventional detection for detecting pathogenic microorganisms in lower respiratory tract multidrug-resistant bacterial infections [n (%)]

Pathogens	mNGS test (n = 279)	Conventional test (n = 121)
Bacteria		
Klebsiella pneumoniae	40 (14.34)	16 (13.22)
Acinetobacter baumannii	32 (11.47)	14 (11.57)
Staphylococcus aureus	28 (10.04)	12 (9.92)
Pseudomonas aeruginosa	26 (9.32)	11 (9.09)
Enterococcus faecium	22 (7.89)	10 (8.26)
Escherichia coli	15 (5.38)	7 (5.79)
Stenotrophomonas maltophilia	10 (3.58)	5 (4.13)
Proteus mirabilis	9 (3.23)	4 (3.31)
Streptococcus pneumoniae	9 (3.23)	5 (4.13)
Corynebacterium striatum	3 (1.08)	1 (0.83)
Morganella morgenia	2 (0.72)	1 (0.83)
Staphylococcus haemolyticus	2 (0.72)	1 (0.83)
Klebsiella aerogenes	2 (0.72)	1 (0.83)
Viruses		
Circovirus	15 (5.38)	6 (4.96)
Human herpesvirus type 1 (HSV1)	7 (2.51)	3 (2.48)
Human herpesvirus type 4 (EBV)	6 (2.15)	3 (2.48)
Human herpesvirus type 5 (CMV)	6 (2.15)	2 (1.65)
Human herpesvirus type 7	5 (1.79)	2 (1.65)
SARS-COV-2	3 (1.08)	2 (1.65)
Influenza A virus H1N1	3 (1.08)	1 (0.83)
Human respiratory virus type 3	2 (0.72)	1 (0.83)
Fungi		
Candida albicans	14 (5.02)	6 (4.96)
Aspergillus fumigatus	7 (2.51)	3 (2.48)
Pneumocystis jirovecii	1 (0.36)	0
Candida subsmoothens	2 (0.72)	1 (0.83)
Candida tropicalis	2 (0.72)	1 (0.83)
Special Pathogens		
Mycobacterium tuberculosis	5 (1.79)	2 (1.65)
Chlamydia psittaci	1 (0.36)	0

Multivariate analysis of multidrug-resistant bacterial infections in the lower respiratory tract. Taking MDRO infection as the dependent variable, the variables associated with MDRO infection and univariate analysis were set as independent variables, and the risk factors were analyzed by Logistic regression. Four variables were substituted into the regression model with $\alpha = 0.05$ as the level, namely, the time of stay in the RICU was ≥7 days, the application of more

Table 1. Comparison of Pathogen Detection Time and Detection Rate between mNGS Testing and Conventional Testing in Patients with Multidrug-Resistant Bacterial Infections of the Lower Respiratory Tract [±s), n (%)]

Method	Number of cases	Pathogen time (h)	Detection detected [cases (%)]	Not detected [cases (%)]
mNGS Test	95	50.76 ± 1.730	85 (89.47)	10 (10.53)
Conventional Test	95	55.53 ± 2.782	64 (67.37)	31 (32.63)
t/χ^2		14.185		13.716
P		0.000		0.000

Table 3. Univariate analysis of multidrug-resistant bacterial infections in patients with lower respiratory tract infections n (%)

Factors	Non-MDRO Group (n = 150)	MDRO Group (n = 95)	x^2	P
Gender			0.710	0.399
Male	85 (56.67)	59 (62.11)		
Female	65 (43.33)	36 (37.89)		
Age (years)			0.239	0.625
>70 years	90 (60.00)	54 (56.84)		
≤70 years	60 (40.00)	41 (43.16)		
Smoking			2.608	0.106
Yes	79 (52.67)	60 (63.16)		
No	71 (47.33)	35 (36.84)		
RICU Stay Duration			8.633	0.003
≥7d	52 (34.67)	51 (53.68)		
<7d	98 (65.33)	44 (46.32)		
Use of more than 2 antibiotics			14.960	0.000
Yes	38 (25.33)	47 (49.47)		
No	112 (74.66)	48 (50.53)		
Diabetes			2.058	0.151
Yes	68 (45.33)	52 (54.74)		
No	82 (54.67)	43 (45.26)		
Invasive procedures			36.645	0.000
Yes	51 (34.00)	70 (73.68)		
No	99 (66.00)	25 (26.32)		
Duration of invasive mechanical ventilation			13.691	0.000
≥7d	45 (30.00)	51 (53.68)		
<7d	105 (70.00)	44 (46.32)		

than 2 antibiotics, invasive operations, and the time of mechanical ventilation was ≥7 days. The results of multivariate Logistic regression analysis of MDRO infection are shown in Table 4.

The impact of mNGS test results on treatment regimens against infection. 95 patients with multidrug-resistant bacteria infection in the lower respiratory tract were given empirical anti-infective treatment before mNGS testing, and finally 85 patients were adjusted according to the results of mNGS testing. Among 95 patients with multidrug-resistant bacteria in the lower respiratory tract, 25 had negative microbial cultures from bronchoalveolar lavage fluid in two consecutive samples (with a sampling interval of ≥24 h), and were therefore released from contact isolation. Results: 75 cases improved and were discharged, 8 cases were ineffective, and 12 cases died.

DISCUSSION AND CONCLUSIONS

Multidrug-resistant organisms (MDRO), mainly refer to bacteria that are resistant to three or more types of antibacterial drugs used in clinical use at the same time. Lower respiratory tract infection is one of the important risk factors affecting the prognosis of RICU patients, and multidrug-resistant bacteria infection further increases the mortality rate of critically ill patients. In recent years, with the irregular use and even abuse of antibiotics, bacterial resistance has become increasingly prominent, and multidrug-resistant bacteria have become important pathogens of hospital infections. Early identification of pathogens, optimization of antibiotic use, and avoidance of antibiotic abuse are crucial to patient prognosis [7, 8]. Routine microbial culture is currently a common diagnostic basis, but the positive rate of culture is low. mNGS does not rely on known nucleic acid sequences and does not require special probe design. Based on polymerase chain reaction, mNGS can directly detect unknown pathogenic microorganisms. It has the characteristics of high throughput, unbias, and short detection time, breaking the limitations of traditional microbial testing [9–13]. In this study, the detection time of pathogens detected by mNGS was (50.76 ± 1.730) h, which was significantly shorter than that of conventional detection and culture (55.53 ± 2.782) h. mNGS technology can provide fast and accurate pathogen detection results, which is helpful for early diagnosis and treatment decisions in patients with multidrug-resistant bacterial infections in the lower respiratory tract.

In this study, among 95 patients with multi-drug resistant bacteria infection in the lower respiratory tract, the positive rate of alveolar lavage fluid mNGS was 89.47%, while the positive rate of routine testing was 67.37%. 26 cases were negative in routine testing, while mNGS was positive, and the positive rate of mNGS was higher than that of routine testing. A total of 279 pathogens were detected by mNGS technology, and 121 pathogens were detected by routine testing. There was no statistical difference in the detection of bacteria, viruses, fungi and special pathogens between the two, but the number of pathogenic microorganisms detected by mNGS was significantly increased compared with conventional testing. It is suggested that

Table 4. Multivariate logistic regression analysis of multidrug-resistant bacterial infections in patients with lower respiratory tract infections

Factors	β value	SE	$Wald\chi^2$ value	P value	OR	95%CI
RICU Stay Duration ≥7d	0.326	0.132	9.562	0.012	1.917	1.023–3.214
Use of More Than 2 Antibiotics	0.756	0.165	24.120	0.000	3.256	2.561–9.378
Invasive Procedures	0.489	0.142	10.096	0.003	2.267	1.421–4.523
Duration of Invasive Mechanical Ventilation ≥7d	0.574	0.154	11.114	0.002	2.496	1.665–5.694

mNGS can help to discover new bacteria, fungi and viruses that are difficult to detect by routine testing, especially the detection of special pathogens. In this study, mNGS detected Pneumocystis yersini and Chlamydia psittacus, but failed to detect it with routine testing, which can prompt clinicians to use precise drugs to avoid ineffective antibiotics. In patients whose pathogens cannot be detected in routine culture, alveolar lavage fluid mNGS can be used as an effective detection method to guide the application of antimicrobial drugs, reduce the production of multi-drug resistant bacteria, and reduce the risk of multi-drug resistant bacteria transmission.

Lower respiratory tract infections in RICU patients are often mixed infections of multiple pathogens and multi-drug resistance. In this study, it was found that the largest number of *K. pneumoniae* was detected in mNGS and routine testing. *K. pneumoniae* is one of the common pathogens of lower respiratory tract infections. Due to multiple organ failure, low immunity and repeated use of a variety of broad-spectrum antibiotics, especially carbapenem antibiotics in RICU patients, the detection of *K. pneumoniae* increased. The second largest number of detections was *Acinetobacter baumannii*, which can enter patients through invasive procedures such as respiratory tract, skin mucosa and tracheal intubation, bronchoscopy suction, and invasive respirator use. In patients who have undergone tracheal intubation, used invasive ventilators and taken a large amount of broad-spectrum antibiotics, especially those with diabetes mellitus, their body's defense function is weakened and the balance of the flora is disrupted. As a result, *Enterococcus faecium*, *Escherichia coli* and *Proteus mirabilis*, which originally present in the intestine, can easily break through the defense barrier of the respiratory tract of the body and enter the respiratory tract to cause infections. In this study, a certain number of *E. faecium* or *E. coli* or *P. mirabilis* were detected by metagenomic next-generation sequencing (mNGS) in the bronchoalveolar lavage fluid specimens. Meanwhile, these patients showed symptoms related to respiratory tract infections such as fever, cough, expectoration and chest pain, and imaging examinations indicated pulmonary inflammation. According to medical records, clinicians adjusted the use of antibiotics based on the results of drug susceptibility tests. The pathogens detected by routine testing and culture were then tested for drug sensitivity, and most of the bacteria were identified as multi-drug resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant antibiotics Enterobacteriaceae (CRE), carbapenem-resistant antibiotics *A. baumannii* (CR-AB), multi-drug-resistant/pan-drug-resistant *Pseudomonas aeruginosa*, etc.

Multivariate logistic regression analysis suggested that the use of more than two antibiotics increased the risk of multi-drug resistant bacteria infection in patients with lower respiratory tract infections, which was the most important risk factor. The intensity of drug use was positively correlated with the infection rate of multi-drug resistant bacteria, and the unreasonable use and abuse of antibiotics could lead to increased bacterial resistance [14]. A variety of antibacterial drugs easily disrupt the micro-ecological balance of the human body. After the sensitive strains are killed, airway bacteria colonize, resulting in the destruction of the healthy flora of the oropharynx, Gram-negative bacteria begin to grow rapidly, and the flora is dysregulated, which increases the probability of MDRO infection in the lower respiratory tract [15]. mNGS is one of the key technologies to achieve precise treatment of infectious diseases and avoid the abuse of antibiotics [16]. Clinicians should choose antimicrobials rationally based on the results of clinical microbial tests, minimize the combination of drugs, and reduce the incidence of MDRO infection in patients with lower respiratory tract infections [17].

Invasive procedures are a risk factor for multidrug-resistant bacterial infections in the lower respiratory tract. Patients in the RICU ward are in critical condition. Most patients require invasive procedures such as mechanical ventilation, central venous intubation, and indwelling urinary catheters. There are many patients with tracheotomy and tracheal intubation. These operations destroy the respiratory barrier. Coupled with the influence of factors such as poor immunity of patients, it is easy to develop respiratory multidrug-resistant bacterial infections/colonization, which leads to more complex conditions and increases the difficulty of clinical treatment. Healthcare workers should strictly implement aseptic technical operations and standard operating procedures when performing invasive operations to avoid contamination.

Invasive mechanical ventilation time ≥7 days is a risk factor for lower respiratory tract multidrug-resistant bacterial infection. The longer the invasive mechanical ventilation time, the easier it is to increase the multidrug-resistant bacterial infection in the lower respiratory tract. Clinicians should strictly grasp the indication of tracheal intubation, evaluate whether the machine can be withdrawn or extubated every day, remove the tracheal intubation as soon as possible, and try to use a mask for continuous positive airway pressure mechanical ventilation, which can effectively prevent lower respiratory tract multidrug-resistant bacterial infection [18].

Patients staying in the RICU for more than 7 days is a risk factor for multi-drug resistant bacteria infection in the lower respiratory tract. The analysis of the reasons is that the RICU environment layout is unreasonable, the indoor ventilation conditions are not good, and the longer the patient stays in the RICU, the longer the time they are exposed to the multi-drug resistant bacteria environment, and the risk of infection increases accordingly. In addition, the personal flow in the RICU is large, and medical instruments and equipment are easily contaminated. Frequent contact between patients and between patients and medical equipment can lead to the transmission of pathogens through the hands of medical staff or shared equipment. Therefore, shortening the time patients stay in the RICU can reduce the incidence of multi-drug resistant bacteria infection in the lower respiratory tract.

After the department implemented various prevention and control measures for multidrug-resistant bacteria and adjusted the antimicrobial drugs according to the mNGS test results, 95 patients with multidrug-resistant bacteria infection in the lower respiratory tract and 25 patients with alveolar lavage fluid were treated twice in a row (sampling time interval ≥24 h), and the microbial culture was negative, so they were released from contact isolation.

This study has the following limitations: ① This study is a retrospective study, with the possibility of selection bias and confounding factors; ② mNGS detected a variety of pathogens in BALF samples from patients with multidrug-resistant bacterial infections in the lower respiratory tract, but could not directly detect multidrug-resistant bacteria, which still needs to be determined in combination with drug susceptibility tests. ③ The cost of mNGS testing is high, and clinicians do not perform mNGS testing again to evaluate the efficacy after adjusting antibacterial drugs according to the results of mNGS testing.

To sum up, mNGS has a good detection efficiency in patients with multi-drug resistant bacterial infections in the lower respiratory tract, and can quickly detect more pathogens. Clinicians should pay special attention to high-risk patients with risk factors while treating patients' diseases. When controlling the time of admission to the RICU, using antibiotics rationally, and performing invasive operations, sterile technical operations should be strictly implemented, cleaning, disinfection, and isolation should be strengthened, and hand hygiene should be strictly enforced. Patients with invasive mechanical ventilation should be withdrawn or extubated as soon as possible. Through the comprehensive application of these measures, the occurrence and spread of multi-drug resistant bacterial infections can be effectively reduced, and the treatment effect of patients can be improved.

Compliance with ethical standards: This study has been approved by the Medical Ethics Committee of First Affiliated Hospital of Guangdong Pharmaceutical University (Ethical approval number is: Medical Ethics Review [2024] IIT No. (52). Patients were consented by an informed consent process that was reviewed by the Ethics Committee of First Affiliated Hospital of Guangdong Pharmaceutical University and certify that the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki.

Conflict of interest: The authors state no conflict of interest with respect to the research, authorship, and/or publication of this article.

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