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Study on the application of microfluidic-based in vitro diagnostic technology in pathogenic detection of respiratory tract infections

Jianping Jiang[1†], Yunqi Wei[1,2†], Shumin Li[1], Juanfen Mo[3], Xiaosi Li[4], Mengqing Cao[1*] and Haiqin Wang[1,2*]

Abstract

Objective To investigate the clinical application value of microfluidic-based in vitro diagnostic (IVD) technology in pathogenic detection of respiratory tract infections.

Methods A total of 300 clinical samples, including blood, bronchoalveolar lavage fluid, and pleural effusion, were collected from patients with respiratory tract infections. The samples were randomly divided into three groups: A, B, and C, with 100 cases in each group. Group A used traditional microbiological detection methods, Group B used metagenomic next-generation sequencing (mNGS) technology, and Group C used both microfluidic-based IVD technology and traditional microbiological detection methods to detect pathogenic microorganisms in the clinical samples. The positive detection rate, detection time, and detection cost were compared among the groups. The diagnostic performance of each group was compared using the Receiver Operating Characteristic (ROC) curve.

Results Traditional microbiological detection identified 38 positive samples (38%), including 45 pathogens; mNGS technology identified 95 positive samples (95%), including 210 pathogens; microfluidic-based IVD technology identified 96 positive samples (96%), including 158 pathogens. Microfluidic-based IVD technology had a significantly higher positive detection rate for pathogenic microorganisms compared to traditional culture techniques (96% vs 38%, $\chi 2 = 122.0$, $P < 0.01$), and it was also faster and cheaper than mNGS technology. ROC analysis showed that compared to traditional microbiological culture results, microfluidic-based IVD technology had significantly increased sensitivity and specificity, similar to mNGS technology.

Conclusion In respiratory infectious diseases, microfluidic-based IVD technology had a higher detection rate for pathogenic microorganisms than traditional culture methods, and it had advantages in detection time and cost compared to mNGS technology. It could also detect critical drug-resistant genes of pathogens. Hence, microfluidic-based IVD technology can be a viable option for diagnosis and treatment of respiratory infectious diseases.

Keywords Respiratory tract infection, Pathogenic detection, Traditional microbiological detection, Metagenomic next-generation sequencing technology, Microfluidic-based in vitro diagnostic technology, Diagnostic performance

†Jianping Jiang and Yunqi Wei contributed equally to this work.

*Correspondence:
Mengqing Cao
caomengqing_0701@163.com
Haiqin Wang
w_haiqin@163.com
Full list of author information is available at the end of the article

Introduction

Infectious diseases pose a serious threat to human health, with a global incidence rate on the rise and pathogens showing a trend towards diversification and complexity [1]. Various newly emerging and re-emerging infectious diseases, difficult-to-detect multiple infections, and fevers of unknown origin pose a significant threat to human health. Respiratory tract infections are particularly important among infectious diseases, being common and prevalent in clinical practice, and also a major factor contributing to morbidity and mortality in infants, children, the elderly, and immunocompromised patients. Therefore, higher requirements have been put forward for the accuracy and effectiveness of diagnosis of respiratory infectious diseases in clinical practice.

There are multiple diagnostic methods for pathogens. Traditional microbiological detection method is simple to operate, technically mature, and has high positive predictive value, and still plays an important role in the identification of pathogens [2]. Microbial isolation and culture rely on the vitality of the pathogens, but a considerable number of pathogens cannot be detected through in vitro culture, resulting in low positive rate, long cycle, and low accuracy [3]. Metagenomic next-generation sequencing (mNGS) technology has demonstrated promising applications in many fields and has been increasingly used in the field of clinical infectious diseases [4–6]. mNGS technology combines high-throughput sequencing and bioinformatics analysis to directly detect all nucleic acids in the sample, and then align them with the reference genome to determine the species and abundance of all known microorganisms in the sample [7]. mNGS technology can detect unknown pathogens in a non-targeted and one-time manner, with a wider detection range and higher accuracy. However, mNGS technology is limited by complex operation procedures, high cost, and susceptibility to interference from human nucleic acids [8]. Therefore, the rapid and accurate detection of pathogens, broad-spectrum screening, and precision medicine have become urgent and necessary tasks in the contemporary world.

Microfluidic-based IVD technology devices are widely used for molecular biology, chemical and biochemical analysis. Microfluidic technology enables detection and fluid regulation in one single component, that increased sensitivity and specificity to detect target analytes at small volumes overcomes several challenges encountered while using traditional diagnostics [9]. Microfluidic based a chip for simultaneous detection of multiple pathogens is a sensitive, specific and an easy to use tool for disease detection as it requires a small volume of sample to amplify the target DNA/RNA [10]. Over the last decade, microfluidic-based IVD technology has been widely accepted as a rapid and an economical form of diagnostic tool as compared to traditional laboratory-based testing [11]. Several studies have shown that use of microfluidic-based IVD technology reduce overall per patient cost, length of stay in hospitals and provide faster results as compared to a traditional laboratory testing [12, 13]. Currently, microfluidic-based in vitro diagnostic (IVD) kits for respiratory tract infections play a crucial role in respiratory tract pathogen detection technology. The maturity and clinical application of this technology can provide more favorable and precise treatment for a large number of patients with respiratory tract infections. In this study, microfluidic-based IVD technology was compared with traditional microbiological detection methods and mNGS technology to determine the presence of pathogens in different target samples and explore the clinical application value of microfluidic-based IVD technology in pathogenic detection of respiratory tract infections.

Methods

Patients

A total of 300 patients (160 males and 140 females) with respiratory tract infections admitted to the Department of Respiratory and Critical Care Medicine of The Second hospital of Jiaxing from January 2022 to December 2022 were included. The average age of all patients was 60.5 ± 16.1 years. The patients were randomly divided into three groups: A, B, and C, with 100 cases in each group.

Diagnostic criteria for inclusion cases (diagnosis can be made if either of the following two criteria is met): (1) Patients present with cough, expectoration, wet rales in the lungs, and one of the following conditions: Fever; Increased white blood cell count and/or neutrophil percentage; Chest X-ray or CT scan suggesting inflammatory infiltrative lesions in the lungs; (2) Stable period of chronic airway diseases (chronic bronchitis with or without obstructive emphysema, asthma, bronchiectasis) with secondary acute infection, accompanied by microbiological changes or significant changes or new lesions in imaging compared to before admission.

Sample collection

Bronchoalveolar lavage fluid (BALF): BALF was obtained from the patient during fiberoptic bronchoscopy. 10 ml of BALF was placed in a sterile container and stored at 4 °C.

Pleural effusion: Patients with pleural effusion were collected by thoracentesis under ultrasound-guided local anesthesia for 10 mL, and 10 ml of pleural effusion was placed in a sterile container and stored at 4 °C.

Blood: Suitable peripheral veins should be disinfected locally, and the first set of blood cultures should be collected using a blood lancet. The second set of blood cultures should be collected from another site using the same method. A volume of 10 mL of blood was collected from each vial and promptly sent for testing.

After collecting the three types of specimens, they should be sent for testing as soon as possible within 2 h at room temperature. If it cannot be sent for testing in a timely manner, BALF and pleural effusion specimens should be stored at 4 °C and sent for testing within 24 h. For long-term storage, they should be stored in a − 80 °C freezer. Refrigerating or freezing blood culture specimens must be avoided.

Testing methods

Group A samples were tested using traditional microbiological detection methods, following the traditional microbiological standard testing method of "inoculation-cultivation-identification-antimicrobial susceptibility". Specific process: The specimen was plated into blood agar plates, chocolate agar plates, and Sabouraud agar plates, and incubated for 24 h. Suspected colonies were subjected to Gram staining and identified to species using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

Group B samples were tested using mNGS technology by Innovative Diagnosis and Treatment (Hangzhou, China). First, DNA was extracted using the TIANamp Micro DNA Kit (DP316; Tiangen Biotech, Beijing, China) according to the manufacturer's instructions, and DNA libraries were constructed based on the sequencer-100 developed by the Beijing Institute of Genomics. Then, high-quality sequencing data was generated by removing low-quality, adapter-contaminated, duplicate, and short (< 35 bp) reads. Next, the high-quality data was mapped to the human genome (hg19; https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.13/) using the Burrows-Wheeler Alignment (BWA; http://bio-bwa.sourceforge.net/) method, and the annotated human genome data was extracted [14]. The reference database for classification was downloaded from NCBI (ftp://ftp.ncbi.nlm.nih.gov/genomes/).

The C group samples were tested using a microfluidic chip kit (Innovative Diagnosis and Treatment, Hangzhou, China). This test kit could simultaneously detect 158 respiratory tract pathogens. According to the kit's instructions, the sample was pre-treated and then centrifuged for rapid DNA extraction. DNA extraction procedure: 500 μL of blood sample was transferred from the vacutainer to an eppendorf tube. Then centrifugated the sample at 3000 RCF for 10 min at 4 °C; 1 mL of RLB was added to the precipitate, mixed gently. The supernatant was discarded and repeated 1–2 times until a white colored pellet is obtained. 500 μL of prewarmed DNA extraction buffer was added to the pellet and set aside. 500 μL isoamylalcohol was added to the mixture after incubation and shaken well and centrifuged. A white precipitate was precipitated by adding 1 mL anhydrous ethanol. Repeated 3 times, ratio of the absorbance at 260 and 280 nm respectively (A260/A280) were estimated to check the quality and quantity of the extracted DNA sample.

After mixing the sample with the isothermal amplification reagent, loop-mediated isothermal amplification (LAMP) was performed (50 min). After the detection was completed, the computer automatically performed data analysis, and the total process time was approximately 1–4 h.

Positive diagnostic criteria

Positive microbiological culture identification results are usually based on the growth and identification of microorganisms, including morphological observations, biochemical reactions and, if necessary, molecular biological confirmation.

The positive diagnostic criteria for mNGS referred to the "Clinical practice expert consensus for the application of metagenomic next generation sequencing" [15], which comprehensively evaluates the frequency of pathogen occurrence, clinical pathogenicity, the number of detected reads, and the relative abundance of the species in the sample. The relative abundance value, which refers to the proportion of the microorganism detected in the entire specimen, is considered positive if it is greater than 1%.

The positive diagnostic criteria for microfluidic-based IVD technology refer to the national standard GB/T41521-2022 "General technical requirement of microfluidic chip for multi-index nucleic acid isothermal amplification and detection", where the detection results are considered positive if they match the positive reference or positive standard, and negative if they show other detection indicators.

Statistical analysis

The data were analyzed using GraphPad Prism 9.0 statistical software. The Kolmogorov–Smirnov method was used for normality tests, with $P > 0.1$ indicating a normal data distribution. Continuous variables with normal distribution were represented by mean ± standard deviation (mean ± SD), and data were compared by independent sample *t*-test. The not-normal data were analyzed using chi-square ($\chi 2$) test or Fisher's exact test. $P < 0.05$ is considered statistically significant. The receiver operating

characteristic (ROC) curve was plotted, and the area under the curve (AUC) was calculated.

Results

Comparison of clinical characteristics of patients

A total of 300 patients with respiratory tract infections were included in this study from January 2022 to December 2022. The patients were randomly divided into three groups, A, B, and C, with 100 people in each group. Group A used traditional microbiological detection methods, Group B used mNGS technology, and Group C used microfluidic-based IVD technology. All three groups collected BALF, pleural effusion, and blood for microbiological detection. The average age of patients in Group A was 62.50 ± 4.98 years, in Group B was 59.94 ± 2.61 years, and in Group C was 58.67 ± 2.42 years. The most common symptoms were cough/expectoration and wet rales, followed by fever and chest tightness/chest pain and dyspnea. All patients detedted white blood cell count, neutrophil percentage, C-reactive protein, and procalcitonin values. The specific values are shown in Table 1. There were no significant difference in the clinical characteristics among the three groups, indicating the comparability of the research results.

Table 1 Clinical characteristics of patients in three groups

Group	A (n = 100)	B (n = 100)	C (n = 100)	*P*
Sex (n)				0.98[a]
Male	59	59	60	
Female	41	41	40	
Average age (mean ± SD)	62.50 ± 4.98	59.94 ± 2.61	58.67 ± 2.42	0.57[a]
≥ 60 years (n)	67	60	62	
< 60 years (n)	33	40	38	
Clinical symptoms (n, %)				
Cough/expectoration	88	89	92	0.63[a]
Wet rales	59	55	64	0.43[a]
Fever	37	29	34	0.47[a]
Chest tightness/chest pain	32	28	32	0.77[a]
Dyspnea	5	2	3	0.48[b]
Laboratory examination results				
White blood cell count (10^9/L)	7.43 ± 0.84	7.56 ± 0.91	7.47 ± 1.02	0.78
Neutrophil percentage	72.3 ± 8.73	74.5 ± 5.49	74.9 ± 7.31	0.72
C-reactive protein (mg/L)	5.92 ± 0.98	6.24 ± 1.03	6.17 ± 1.21	0.66
Procalcitonin (ng/mL)	4.81 ± 0.77	4.52 ± 0.68	4.42 ± 0.59	0.89
Sample types				0.62[b]
Bronchoalveolar lavage fluid	86	86	90	
Pleural effusion	8	11	8	
Blood	6	3	2	

a: chi-square test, b: Fisher's exact test. *, $P < 0.05$

Comparison of pathogen positive detection rates

To detect samples from patients with respiratory infectious diseases, we compared and analyzed the positive detection rates of pathogenic microorganisms by traditional microbiological detection methods, mNGS technology, and microfluidic-based IVD technology. As shown in Table 2, the pathogen positive rate in Group A was 38%, in Group B was 95%, and in Group C was 96%. The positive detection rates showed significant differences ($P < 0.01$). In addition, the positive rates in Group B and Group C were significantly higher than that in Group A ($P < 0.001$), while there was no significant difference between Group B and Group C ($P > 0.05$, Fig. 1).

Subsequently, comparative analysis was conducted based on the types of detection samples. As shown in Table 3, the positive detection rates of pathogenic microorganisms in Group A were 39.5%, 25.0%, and 33.3%

Table 2 Comparison of pathogen positive detection rates among three groups

	Positive (n)	Negative (n)	Positive detection rate (%)	χ^2	*P*
Group A	38	62	38	122.0	< 0.01
Group B	95	5	95		
Group C	96	4	96		

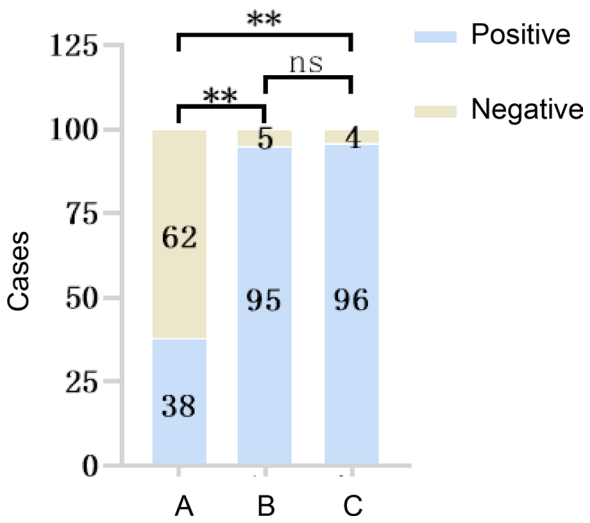

Fig. 1 Pathogen positive detection rates in three methods. **A** represents detection by traditional microbiological methods; **B** represents detection by mNGS technology; **C** represents detection by microfluidic-based IVD technology. The data were analyzed using chi-square ($\chi 2$) test. **$P < 0.01$

Table 3 Comparison of pathogen positive detection rates among three types of samples

	A (n, %)	B (n, %)	C (n, %)
Bronchoalveolar lavage fluid	34 (39.5)	83 (96.5)	86 (95.6)
Pleural effusion	2 (25.0)	9 (81.8)	8 (100)
Blood	2 (33.3)	3 (100)	2 (100)
c^2/P	0.72/0.70	4.60/0.10	0.46/0.79

Table 4 Pathogen detection results

	A	B	C	*P*
Bacteria	28	154***	99***,##	< 0.01
Fungi	17	22*	20*	< 0.01
Virus	0	20***	22***	0.02
Atypical pathogen	0	14***	17***	0.04

Compared with Group A, * indicates $P < 0.05$ and *** indicates $P < 0.001$; compared with Group B, ### indicates $P < 0.01$

respectively. The positive detection rates of pathogenic microorganisms in Group B were 96.5%, 81.8%, and 100% respectively. The positive detection rates of pathogenic microorganisms in Group C were 95.6%, 100%, and 100% respectively. There were no statistically significant differences within three groups. Furthermore, there were significant differences in the positive detection rates among the three groups ($P < 0.01$). Among them, the positive detection rates of Group B and Group C were significantly higher than Group A ($P < 0.01$). However, there was no significant difference between Group B and Group C ($P > 0.05$).

Pathogen detection results

As shown in Table 4, an analysis was conducted on the types of pathogens detected in the three groups in summary. The results revealed that only bacteria and fungi were detected in Group A. In addition to bacteria and fungi, viruses and atypical pathogens were also detected in groups B and C. Among the four types of pathogens, the detection rates in groups B and C were significantly higher than in group A ($P < 0.01$). The detection rate of bacteria in group B was higher than in group C ($P < 0.01$), but there was no significant difference between groups B and C in detecting other types of pathogens ($P > 0.05$).

The identified pathogens were classified. A total of 45 pathogens were detected in group A, including 28 bacteria (Fig. 2A) and 17 fungi (Fig. 2B). The most common bacteria species were *Pesudomonas aeruginosa* (5, 17.9%), followed by *Actinetobacter baumannii* (4, 14.3%), *Klebsiella pneumoniae* (3, 10.7%), *Mycobacterium tuberculosis* (3, 10.7%), and *Corynebacterium striatum* (3, 10.7%). The most common fungal species was *Candia albicans* (12, 70.6%), followed by *Aspergillus fumigatus* (4, 23.5%).

A total of 210 pathogens were detected in group B, including 154 bacteria (Fig. 3A), 22 fungi (Fig. 3B), 20 viruses (Fig. 3C), and 14 atypical pathogens (Fig. 3D). Among them, the most common bacterial species were *Mycobacterium tuberculosis* (19, 12.3%) and *Pesudomonas aeruginosa* (12, 7.8%), followed by *Klebsiella pneumoiae* (9, 5.8%), *Staphylococcus aureus* (9, 5.8%), *Haemophilus influenzae* (8, 5.2%) and *Streptococcus constellatus* (8, 5.2%). The most common fungal species was *Candida albicans* (6, 27.3%), followed by *Aspergillus fumigatus* (4, 18.2%). The most common virus species were *Human gammaherpesvirus 4* (8, 40.0%), followed by *Human alphaherpesvirus 1* (4, 20.0%), *Human betaherpesvirus 5* (3, 15.0%), and *Torque teno virus* (2, 10.0%). Among the atypical pathogens, the largest proportion was *Chlamydia psittaci* (6, 42.9%), followed by *Mycoplasma pneumoniae* (4, 28.6%) and *Legionella pneumophila* (3, 21.4%).

A total of 158 pathogens were detected in Group C, including 99 strains of bacteria (Fig. 4A), 20 strains of

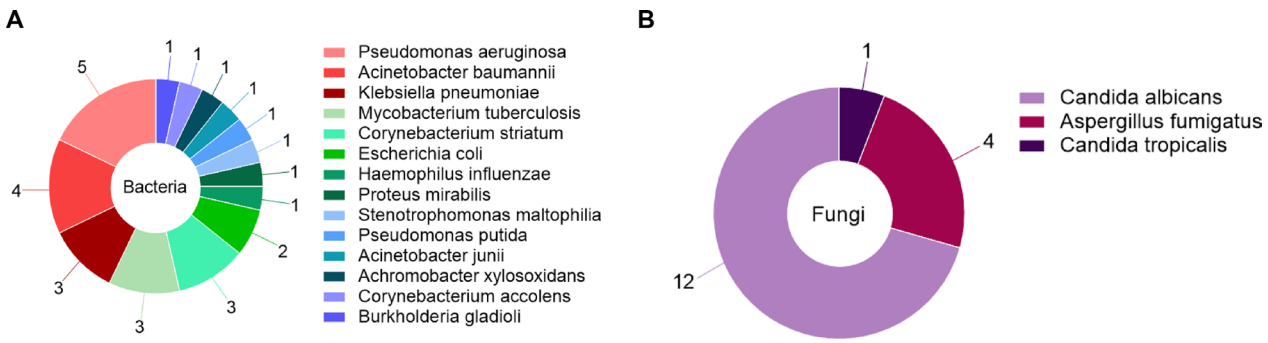

Fig. 2 Bacteria and fungi were detected by traditional microbiological methods. **A** 28 bacteria were detected in group A. **B** 17 fungi were detected in group A

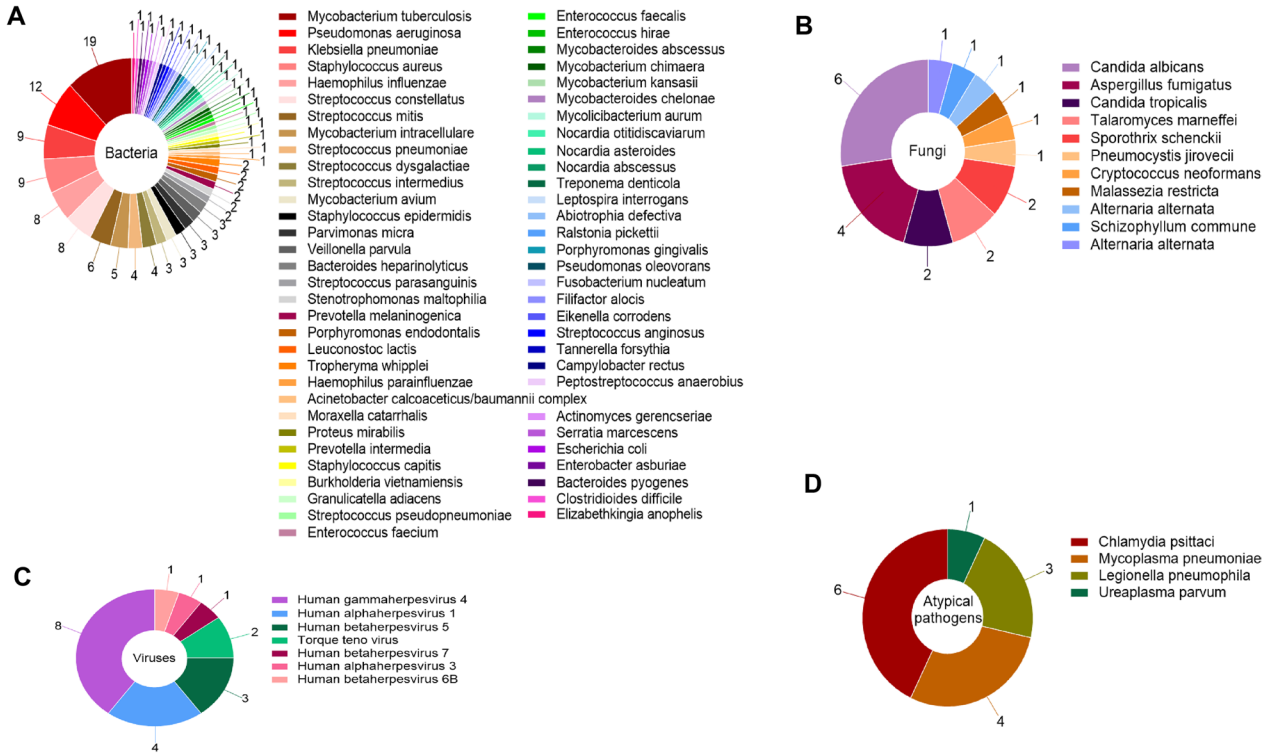

Fig. 3 Bacteria and fungi, viruses and atypical pathogens were detected by mNGS technology. **A** 154 bacteria were detected in group B. **B** 22 fungi were detected in group B. **C** 20 viruses were detected in group B. **D** 14 atypical pathogens were detected in group B

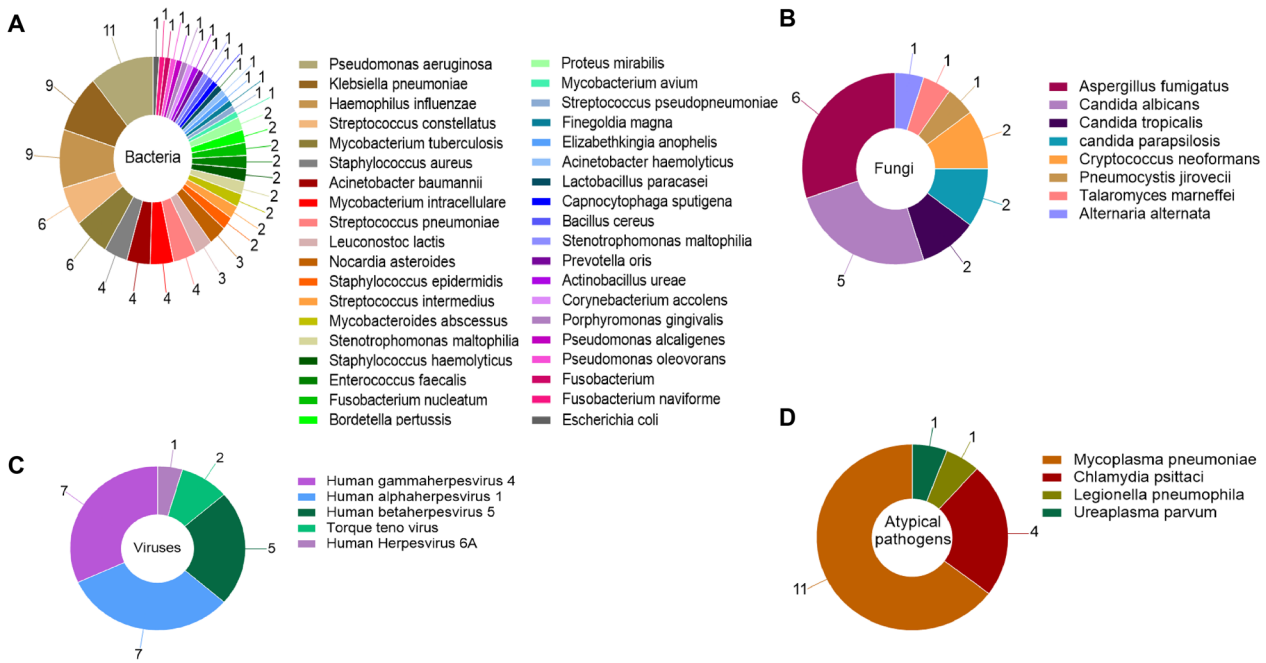

Fig. 4 Bacteria and fungi, viruses and atypical pathogens were detected by microfluidic-based IVD technology. **A** 99 strains of bacteria were detected in group C, **B** 20 strains of fungi were detected in group C. **C** 22 strains of viruses were detected in group C. **D** 17 strains of atypical pathogens were detected in group C

fungi (Fig. 4B), 22 strains of viruses (Fig. 4C), and 17 strains of atypical pathogens (Fig. 4D). The most common bacteria species were *Pesudomonas aeruginosa* (11, 11.1%), followed by *Klebsiella pneumoniae* (9, 9.1%), *Haemophilus influenzae* (9, 9.1%), *Streptococcus constellatus* (6, 6.1%), and *Mycobacterium tuberculosis* (6, 6.1%). The most common fungi species were *Aspergillus fumigatus* (6, 30%) and *Candida albicans* (5, 25%). The most common virus species were *Human gammaherpesvirus 4* (7, 31.8%) and *Human alphaherpesvirus 1* (7, 31.8%), followed by *Human betaherpesvirus 5* (5, 22.7%). The most common atypical pathogen species was *Mycoplasma pneumoniae* (11, 78.6%).

Comparison and analysis of diagnostic performance using three detection methods

ROC curves were used to evaluate the sensitivity and specificity of three detection methods. As show in Fig. 5, the AUC value for group A was 0.6139, with a 95% confidence interval (CI) of 0.5122–0.7156. The AUC value for group B was 0.8839, with a 95% CI of 0.8183–0.9495. The AUC value for group C was 0.8968, with a 95% CI of 0.8394–0.9542. The specific results are shown in Table 5. The sensitivity of group A was 72.30%, the specificity was 55.35%, the positive predictive value was 68.50%, the negative predictive value was 91.66%, the positive likelihood ratio was 2.014, the negative likelihood ratio was 0.116, and the accuracy of the diagnostic test was 61%.

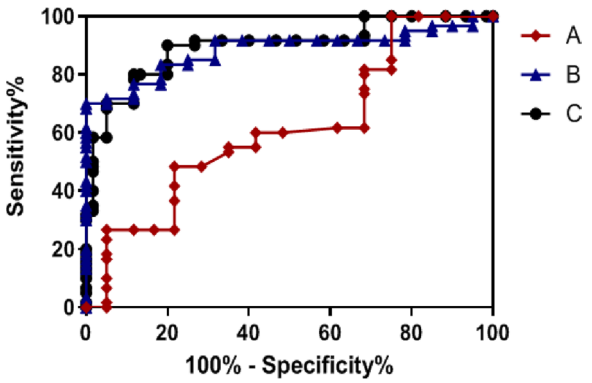

Fig. 5 ROC curves were used to evaluate the sensitivity and specificity of three detection methods

The sensitivity of group B was 92.14%, the specificity was 62.5%, the positive predictive value was 75.14%, the negative predictive value was 95.12%, the positive likelihood ratio was 2.856, the negative likelihood ratio was 0.012, and the accuracy of the diagnostic test was 80%. The sensitivity of group C was 95.12%, the specificity was 66.33%, the positive predictive value was 78.92%, the negative predictive value was 96.85%, the positive likelihood ratio was 3.041, the negative likelihood ratio was 0.125, and the accuracy of the diagnostic test was 82%. The results showed that The results indicated that the predictive value of groups B and C was significantly better than that of group A.

Comparison of detection duration and cost

Finally, we also compared the time and cost required for the three detection methods (Table 6). The results showed that compared with group A, the detection time of group B and group C was significantly shorter ($P<0.001$), but the detection cost of group A was significantly lower than that of group B and group C ($P<0.001$). In addition, the detection duration and cost of group B were significantly higher than those of group C ($P<0.01$).

Discussion

Respiratory tract infections, especially lower respiratory tract infections, are common clinical diseases with high incidence and mortality, and have become the fourth leading cause of death worldwide. In China, respiratory tract infections are also one of the most serious public health problems [16–18]. Bacteria, viruses, fungi, atypical pathogens, and parasites can all cause respiratory tract infections, and the severity of the infection varies. Early rapid detection of respiratory pathogens is crucial for accurate treatment and improving patient prognosis.

Table 6 Duration and cost of different testing methods

	A	B	C	*P*
Duration (h)	98.2 ± 24.3	13.5 ± 0.23***	2.45 ± 0.52$^{***,\#\#\#}$	< 0.01
Cost (Yuan)	127.5 ± 75.85	3500***	1700$^{***,\#\#\#}$	< 0.01

Compared with Group A, *** indicates $P<0.001$; compared with Group B, ### indicates $P<0.001$

Table 5 The ROC curve analysis of three groups

Groups	AUC	95% CI	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR	NLR	Accuracy
A	0.6139	0.5122–0.7156	72.30	55.35	68.50	91.66	2.014	0.116	61.00%
B	0.8839	0.8183–0.9495	92.14	62.50	75.14	95.12	2.856	0.012	80.00%
C	0.8968	0.8394–0.9542	95.12	66.33	78.92	96.85	3.041	0.125	82.00%

AUC, the area under the curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio

Different detection methods have significant differences in the detection rate of respiratory infectious disease pathogens. Traditional microbiological culture methods are time-consuming, usually taking 3–5 days, and negative results require 7 days. Studies have shown that about 70% of infected patients cannot determine the pathogen by taking traditional detection methods, resulting in a lack of timely and effective treatment, leading to worsening of the condition [19]. A study on community-acquired pneumonia in adults showed that only 38% of pathogens could be detected due to low sensitivity of pathogen culture, long time requirements, and limited number of microorganisms detected by serological and PCR tests [14]. Almudena Burillo et al. found that blood culture is not only time-consuming, but also has an overall positive detection rate of pathogenic microorganisms of only 30–40% [20]. In recent years, mNGS technology has gradually matured and has become one of the key technologies for identifying pathogens in respiratory infectious diseases. Chen et al. used mNGS technology to detect pathogens in BALF from patients with lower respiratory tract infections, and the detection rate was significantly higher than that of traditional culture, with a sensitivity of 66.1% and a specificity of 75.4% [18]. Miao et al. compared mNGS technology with traditional culture in the diagnosis of infectious diseases and found that the sensitivity and specificity of mNGS technology were 50.7% and 85.7%, respectively, which were better than traditional culture, especially for *Mycobacterium tuberculosis*, viruses, anaerobic bacteria, and fungi [21]. Quince et al. conducted a study on patients with severe pneumonia, and the results showed that the positive detection rate of pathogenic bacteria by mNGS technology was significantly higher than that of the control group (68.7% vs 45.4%), indicating that mNGS can detect pathogens more quickly and accurately than conventional testing, aid in the adjustment of antibiotics in a timely manner, and significantly reduce the mortality rate of severe pneumonia [22]. These results indicate that compared with traditional culture, mNGS technology improves the detection rate of pathogens, especially for culture-negative clinical samples, and is valuable. However, mNGS technology has difficulties in determining pathogenic bacteria, high cost, and longer detection time (>12 h), making it unable to rapidly detect and monitor in real time. Therefore, there is an urgent clinical need for a new, rapid, and accurate detection method for respiratory pathogens.

This study compared the positive detection rates of three detection methods, traditional microbiological detection methods, mNGS technology, and microfluidic-based IVD technology, for respiratory infectious diseases. The results showed that compared with traditional microbiological detection methods, microfluidic-based IVD technology and mNGS technology had higher specificity and sensitivity. However, microfluidic-based IVD technology had shorter detection time and lower detection cost, indicating that this method had strong advantages in pathogenic detection. In addition, the lack of accuracy in pathogen drug resistance identification is one of the current problems faced by mNGS technology, and clinical doctors need further drug sensitivity culture or drug resistance gene detection for auxiliary diagnosis. Microfluidic-based IVD technology can detect up to 35 drug resistance genes, which has obvious advantages in obtaining drug resistance information of pathogens and helps in the diagnosis and treatment of respiratory infectious diseases.

Although microfluidic-based IVD technology has some advantages, it still faces some problems. Similar to mNGS, there is an issue of varying levels of report interpretation, and clinical judgment needs to be made by combining information such as sample type, microbial characteristics, patient clinical features, traditional microbiological test results, and prior anti-infection treatment. In addition, there may also be problems such as nucleic acid contamination and low nucleic acid extraction levels [23–27]. Therefore, in clinical practice, the combination of traditional detection methods and molecular biology detection methods may help improve the detection rate of pathogens and determine the true pathogens.

The integration of microfluidics has significantly changed disease diagnosis and pathogen detection. The microfluidic enables a high degree of integration of the extraction, amplification and detection processes of nucleic acids, thus improving detection efficiency and accuracy [11]. Microfluidics also enables the detection of tumour-specific biomarkers, thus helping doctors with early diagnosis, efficacy monitoring and prognostic assessment of tumours. Microfluidic chip technology can also be used for the detection of biochemical indicators in blood, urine and other body fluids, such as blood glucose, blood lipids, liver and kidney function [9]. Notably, our study has some limitations. First, each participant has not provided all three types of samples,and that leads to be was variation among participants. Second, the present study was a preliminary study; we will correlate multiple indicators in later experiments, which may further improve the diagnostic efficacy. Based on the results of the current study, we will also conduct further prospective studies to improve the accuracy of diagnosis and treatment of respiratory infectious diseases.

In summary, we used three methods to detect pathogenic microorganisms in 300 clinical samples. Among them, microfluidic-based IVD technology detected pathogenic microorganisms in 96% of respiratory infection cases, with a significantly higher positive detection rate than traditional microbiological culture (38%). It is not only comparable to mNGS technology in the detection rate of pathogenic microorganisms (95%), but also shorter in time and lower in cost. Based on this, the detection of important drug-resistant genes of pathogens can also be achieved based on microfluidic-based IVD technology. Therefore, microfluidic-based IVD technology is a good complementary method to traditional culture methods, and it helps to solve the current clinical dilemma of low pathogen detection rate and reduced efficacy of drug-resistant bacteria and has high clinical application value in the diagnosis and treatment of respiratory infectious diseases.

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Author contributions
JJ and YW dealt with the study experiments and drafted the manuscript. SL and JM performed the experiments and data analysis. XL gave some constructive suggestions for this paper during the revision and production period. MC and HW contributed to conception, design, and critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was conducted in accordance with the Declaration of Helsinki (revised in 2013) and was approved by the Ethics Committee of The Second Hospital of Jiaxing (Ethics number JXEY-2022SW067). Samples were obtained from all patients with written informed consent.

Consent for publication
All patients provided verbal and written consent for publication.

Competing interests
There are no competing interest to declare. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author details
[1]Department of Respiratory Medicine, The Second Affiliated Hospital of Jiaxing University, 1518 Huanchen North Road, Jiaxing 314000, Zhejiang, China. [2]Department of Respiratory Medicine, Jiaxing University Master Degree Cultivation Base, Zhejiang Chinese Medical University, Jiaxing 314000, Zhejiang, China. [3]The Key Laboratory, The Second Affiliated Hospital of Jiaxing University, Jiaxing 314000, Zhejiang, China. [4]Clinical Laboratory, The Second Affiliated Hospital of Jiaxing University, Jiaxing 314000, Zhejiang, China.

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