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Original Article

Diagnostic value of loop-mediated isothermal amplification in detecting lower respiratory pathogens

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Abstract

Introduction: To investigate the diagnostic value of loop-mediated thermostatic amplification (LAMP) in detecting pathogenic bacteria from bronchoalveolar lavage fluid (BALF) of patients with pulmonary disorders combined with lower respiratory tract infections (LRTI).

Methodology: This cross-sectional study included patients with pulmonary disorders combined with LRTI, including chronic obstructive pulmonary disease (COPD), bronchiectasis, or lung cancer, hospitalized in Meizhou People's Hospital between January 2020 and October 2021. BALF was collected using local bronchoalveolar lavage and electronic bronchoscopy. The presence of the pathogens was confirmed using the LAMP method and the bacterial culture method.

Results: In total, 249 patients were included (135 with COPD, 73 with bronchiectasis, and 41 with lung cancer). The proportions of Methicillin-resistant *Staphylococcus aureus* (4.8% vs 0.4%, $p = 0.02$) and *Haemophilus influenzae* (6.8% vs 0.4%, $p < 0.001$) detected by the LAMP method was higher, while the proportion of *Pseudomonas aeruginosa* was lower compared with that of the culture method (6.8% vs 12.4%, $p = 0.034$). The bacterial species with the highest agreement coefficient was *Stenotrophomonas maltophilia* (Kappa = 0.798, $p < 0.001$). Furthermore, 9 COPD patients exhibited mixed infections as determined by the LAMP method, whereas the culture method detected only 2 of these cases (1.48%) ($p < 0.05$).

Conclusions: LAMP can detect more pathogenic bacteria, notably *Haemophilus influenza*, Methicillin-resistant *Staphylococcus aureus*, and atypical pathogens in patients with clinically common pulmonary disorders combined with LRTI. LAMP may provide etiological evidence to guide the clinical use of antibiotics in primary hospitals.

Key words: LAMP; COPD; bronchiectasis; LRTI; lung cancer; pathogenic bacteria.

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Introduction

Chronic obstructive pulmonary disease (COPD), bronchiectasis, and lung cancer are common clinical pulmonary disorders. The number of new cases of these diseases is increasing every year [1]. Respiratory tract infections can alter pulmonary physiology and are recognized as the main cause of lung disease exacerbations [2-4]. Lower respiratory tract infection (LRTI) is the most common type of respiratory infection caused by microorganisms, such as bacteria and viruses [5]. The severity of respiratory infection varies significantly with the type of microorganisms and its treatment depends on the cause of the infection [6]. Therefore, it is necessary to identify the primary pathogen causing the infection to select appropriate treatment strategies for patients with pulmonary disorders.

In general, the diagnostic tests for these multiple types of pathogens include conventional bacterial culture, smear microscopy, biochemical tests, and serological tests [7]. However, these approaches have insufficient diagnostic sensitivity or specificity. It has been reported that PCR amplification can offer significant advantages in terms of accuracy and turnover time [8]. Regrettably, this diagnostic technique is expensive and requires well-equipped facilities. Therefore, it is performed seldom in routine clinical practice. Loop-mediated isothermal amplification (LAMP) is a common technique used for nucleic acid amplification. It is considered rapid, simple, and highly sensitive [9]. In a short period, this technique can amplify specific nucleic acids without expensive laboratory equipment [10]. Recently, LAMP has been widely utilized in a variety of molecular diagnostic applications with the recent improvement in technology. It has been reported that the portable LAMP instruments can be used for early detection of the *X. fastidiosa*, *C. platani*, and *P. ramorum* bacterial species [11]. Moreover, the nucleic acid molecules derived from bacteria, viruses, and parasites have been

previously identified by LAMP [12]. Notably, LAMP method have also been developed for the detection of several respiratory RNA viruses [13]. Bronchoalveolar lavage fluid (BALF) is a sample matrix used in an established bronchoscopic procedure and is recommended for the diagnosis of pulmonary disorders [14]. It can be collected from an infection site with lower risk of contamination by oral bacteria. Therefore, it facilitates the identification of pathogens implicated in the aetiology of involved in respiratory diseases [15], and the characterization of the distinct microbiome in the lower respiratory tract [16].

Nevertheless, a limited number of studies have reported the application of LAMP in detecting pathogenic bacteria in the BALF samples of patients with different pulmonary disorders combined with LRTI. We used the results of the culture method as the reference standard. The present study aimed to investigate the diagnostic value of LAMP in detecting pathogenic bacteria in BALF samples of pulmonary disorders accompanied with LRTI.

Methods

Study design and patients

The cross-sectional study included patients with pulmonary disorders accompanied by LRTI, including COPD, bronchiectasis, or lung cancer, who were hospitalized in Meizhou People's Hospital between January 2020 and October 2021. The inclusion criteria were: 1) Age: 18-100 years old; 2) patient diagnosis compliance with the "*Guidelines for Primary Diagnosis and Treatment of Chronic Obstructive Pulmonary Disease (Practical Edition 2018)*" [17], "*Expert Consensus on the Diagnosis and Treatment of Adult Bronchiectasis (2012 Edition)*" [18], or *Standards for the Diagnosis and Treatment of Primary Lung Cancer in China (2015 Version)* [19]. 2) The patients must have respiratory disease symptoms, such as cough, yellow purulent sputum, severe shortness of breath, hemoptysis, fever, and impaired consciousness; 3) the chest CT or chest X-ray should be indicative of LRTI; 4) the consent of the patient and/or his/her family members was required for the collection of the BALF sample and the respiratory specimens/tissues; 5) the use of sensitive antibiotics should have had a curative effect on the infection treatment or tuberculosis treatment. The exclusion criteria were: 1) Accompanied with severe cardiopulmonary dysfunction, severe hypertension, malignant arrhythmia, coagulation dysfunction, platelet count $< 20 \times 10^9$/L, and patients who were not suitable for electronic bronchoscopy and bronchoalveolar lavage in bronchoscopy; 2) Other diseases that cause serious lung infections, serious infections of other organs, and HIV infections; 3) Uncooperative and refused to be examined; 4) Specimens which did not meet the clinical detection standards. The present study was reviewed and approved by the Medical Ethics Committee of Meizhou People's Hospital. All the participants signed the informed consent form.

Sample preparation and DNA extraction

The bronchoalveolar lavage fluid was collected into a sterile vial according to the routine procedure. For each sample, a parallel study using both routine culture-based and LAMP method was carried out. 1 mL of the supernatant derived from the BALF was transferred into tubes and subsequent concentration by centrifugation at 12,000 rpm for 5 minutes. The supernatant was discarded and precipitate was used for DNA extraction. The extraction of genomic DNA of the bacterial pathogens was performed by using the Universal Kit for Bacterial DNA Extraction Kit (CapitalBio, Chengdu, China) following the manufacturer's recommended protocol. Quality and concentration of the purified DNA samples were measured and evaluated using Nanodrop 2000 TM Spectrophotometer (ThermoFisher Scientific, Waltham, MA) by examining OD260/OD280 and OD260/OD230. The isolated DNA was stored at 20 °C until further use.

Pathogenic bacteria detection

Electronic bronchoscopy was performed on patients following admission according to the operation regulations of "*Guidelines for Diagnostic Flexible Bronchoscopy in Adults (2019 Edition)*" [20] and "*Chinese Expert Consensus on the Pathogens Detection by Bronchoalveolar Lavage in Lung Infectious Diseases (2017 Edition)*" [21]. The nucleic acids of the respiratory pathogenic bacteria were detected in BALF samples by the LAMP method. The pathogenic bacteria were detected by the LAMP method using the nucleic acid detection kit (CapitalBio Corporation, Beijing, China) following the manufacturer's instructions based on a combination of isothermal amplification and the microfluidic chip method. The schematic and the amplification curves for respiratory pathogens detection obtained using the centrifugal force-driven microfluidic chip .The limit of detection for pathogenic bacteria is 500 copies per reaction. Briefly, 20 mL reaction regent and 34.5 mL DNA sample were mixed, and then 50 mL of the mixture was simply added into

microfluidic chip through the distribution channel due to pressure generated by the pipettor using a 200 mL pipette tip. In the process, the air inside the chip escapes through air vents downstream of each reaction well, the inlet ports are covered with tape to prevent contamination. The chip is then placed in a microcentrifuge and followed by centrifugation at 3000 rpm for 30 seconds, then making the mixture drop into the bottom of the reaction wells so as to complete the detection course without cap opening. The reactions were performed on a RTisochipTM-A thermostatic expansion microfluidic chip nucleic acid analyzer, along with the real-time imaging system (CapitalBio Technology, Beijing, China), according to the following protocol: 1 cycles of 3 minutes at 37 °C and 1 cycles of 47 minutes at 65 °C . To visualise the results, respiratory tract pathogen nucleic acid detection software was used to analyze. These experimental methods quoted the article of Dr. Hou of our hospital, and obtained his knowledge and consent [7]. The results of the detection were visualized with the corresponding software and obtained within 24 h. A total of 13 pathogens including *Streptococcus pneumoniae, Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* (PA), *Acinetobacter baumannii, Stenotrophomonas maltophilia, Haemophilus influenzae, Legionella pneumophila, Mycoplasma pneumoniae, Chlamydia pneumoniae*, and *Mycobacterium tuberculosis* were detected. Concomitantly, other bacteria, fungi and Mycobacterium tuberculosis were recovered after treatment and culture of sputum/BALF/tissue samples. According to the routine operation standards for culturing bacteria/fungi used in clinical practice, the specimens were incubated into pre-processed petri dishes and placed in a constant temperature incubator. The bacteria were detected by an automatic bacterial analyzer (VITEK2 BioMerieux, France). Fungal detection was carried out by a fungal analyzer. *Mycobacterium tuberculosis* was detected by liquid-based culture and specific molecular biology methods, such as TB-DNA, *Mycobacterium tuberculosis* nucleic acid test, and Xpert MTB/RI test. Mycobacterium tuberculosis nucleic acid and TB-DNA were detected by biological kit（Daan Gene Co., ltd, Guangzhou, China). Xpert MTB/RI test was made by biological kit (Cepheid AB, Sweden).

Statistical Analysis

The statistical software, SPSS 23.0 (IBM, Armonk, NY, USA) was used for statistical analysis. Continuous variables were presented as mean ± standard deviation (SD). Categorical variables were expressed as frequencies with percentages. The comparison of the proportion for pathogen infection among these 2 methods was conducted using a Chi-square test or Fisher exact test. The result of routine cultures was regarded as the "Gold Standard" .The agreement between LAMP method and bacteria cultures was evaluated by Kappa coefficient (Kappa ≥ 0.75 indicated optimal consistency; 0.75 > Kappa ≥ 0.4, indicated general consistency; Kappa < 0.4 indicated poor consistency). Two-side $p < 0.05$ was considered statistically significant.

Ethics approval and consent to participate

This work has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. This study was approved by Medical Ethics Committee of Meizhou People's Hospital [2020-CY-18], and all participants provided written informed consent.

Results

In total, 249 (180 males and 69 females) patients with pulmonary disorders, including 135 COPD cases (114 males and 21 females), 73 bronchiectasis cases (35 males and 38 females), or 41 lung cancer cases (31 males and 10 females), were included in the present study. Their mean age was 66.14 ± 12.20 years old (Table 1). Among them, 114 patients were smokers, 57 patients had hypertension, 28 patients had type 2 diabetes and 19 patients had coronary heart disease (Table 1).

Table 1. Baseline Characteristics.

Characteristics	COPD (n = 135)	Bronchiectasis (n = 73)	Lung cancer (n=41)	Total (n = 249)
Age	70.41 ± 9.92	60.95 ± 14.22	61.37 ± 9.77	66.14 ± 12.20
Gender (Male/Female)	114/21	35/48	31/10	180/69
Smoking history	88	13	13	114
Hypertension	39	11	7	57
Type 2 diabetes	15	8	5	28
Coronary heart disease	15	2	2	19

Table 2. Comparison of pathogenic bacteria detected by the lamp/culture methods in three groups of patients.

Pathogen, n (%)	The LAMP Method	The Culture Method	*p*
Streptococcus pneumoniae	10 (4.02)	5 (2.01)	0.190
Staphylococcus aureus	6 (2.41)	4 (1.61)	0.523
Methicillin-resistant *Staphylococcus*	12 (4.82)	1 (0.40)	0.002
Escherichia coli	7 (2.81)	4 (1.61)	0.360
Klebsiella pneumoniae	4 (1.61)	3 (1.20)	0.996
Pseudomonas aeruginosa	17 (6.83)	31 (12.45)	0.034
Acinetobacter baumannii	2 (0.80)	1 (0.40)	> 0.999
Stenotrophomonas maltophilia	2 (0.80)	3 (1.20)	> 0.999
Haemophilus influenzae	17 (6.83)	1 (0.40)	< 0.0.01
Legionella pneumophila	2 (0.80)	0 (0.00)	---
Mycobacterium tuberculosis* complex / *Mycobacterium	4 (1.61)	6 (2.41)	0.523
Single infection	62 (24.90)	62 (24.90)	> 0.999
Mixed infections	9 (3.61)	3 (1.20)	0.080

Table 3. Consistency between the LAMP and the culture methods.

Pathogen	The LAMP Method / The Culture Method				Kappa Value	*p*
	+/+	+/-	-/+	-/-		
Streptococcus pneumoniae	4	6	1	238	0.520	< 0.001
Staphylococcus aureus	2	3	2	242	0.434	< 0.001
Methicillin-resistant Staphylococcus	0	12	1	236	---	---
Escherichia coli	4	3	0	242	0.722	< 0.001
Klebsiella pneumoniae	2	2	1	244	0.565	< 0.001
Pseudomonas aeruginosa	15	2	16	216	0.590	< 0.001
Acinetobacter baumannii	0	2	1	246	---	---
Stenotrophomonas maltophilia	2	0	1	246	0.798	< 0.001
Haemophilus influenzae	1	16	0	232	0.104	< 0.001
Legionella pneumophila	0	2	0	247	---	---
Mycoplasma pneumoniae	0	0	0	249	---	---
Chlamydia pneumoniae	0	0	0	249	---	---
Mycobacterium tuberculosis complex	3	1	3	242	0.592	< 0.001

Table 4. Positive Rates of Pathogenic Bacteria Detected by the LAMP and the Culture Methods in Three Diseases combined with LRTI.

	LAMP method	Culture method	*p*
COPD	39 (28.89)	35 (25.93)	0.585
Bronchiectasis	24 (32.88)	25 (34.25)	0.861
Lung cancer	8 (19.51)	5 (12.20)	0.364
Total	71 (28.51)	65 (26.10)	0.546

Table 5. Comparison of Pathogenic Bacteria Detected by the LAMP and Culture Methods in COPD.

Pathogen, n (%)	The LAMP Method (n = 135)	The Culture Method (n = 135)	*p*
Streptococcus pneumoniae	7 (5.19)	1 (0.74)	0.073
Staphylococcus aureus	3 (2.22)	1 (0.74)	0.614
Methicillin-resistant Staphylococcus	8 (5.93)	1 (0.74)	0.042
Escherichia coli	4 (2.96)	3 (2.22)	> 0.999
Klebsiella pneumoniae	3 (2.22)	3 (2.22)	> 0.999
Pseudomonas aeruginosa	7 (5.19)	15 (11.11)	0.075
Acinetobacter baumannii	2 (1.48)	1 (0.74)	> 0.999
Stenotrophomonas maltophilia	2 (1.48)	3 (2.22)	> 0.999
Haemophilus influenzae	11 (8.15)	1 (0.74)	0.003
Legionella pneumophila	2 (1.48)	0 (0.00)	---
Mycobacterium tuberculosis complex / Mycobacterium	1 (0.74)	2 (1.48)	> 0.999
Corynebacterium striatum	0 (0.00)	3 (2.22)	---
Penicillium marneffei	0 (0.00)	1 (0.74)	---
Aspergillus fumigatus	0 (0.00)	2 (1.48)	---
Single infection	30 (22.22)	33 (24.44)	0.666
Mixed infections	9 (6.67)	2 (1.48)	0.031

The proportions of Methicillin-resistant *Staphylococcus aureus* (4.8% vs 0.4%, $p = 0.02$) and *Haemophilus influenzae* (6.8% vs 0.4%, $p < 0.001$) detected by the LAMP method was higher, while the proportion of PA was lower compared with that of the culture method (6.8% vs 12.4%, $p = 0.034$) (Table 2). The bacteria with the largest agreement coefficient was *Stenotrophomonas maltophilia* (Kappa = 0.798, $p < 0.001$). Poor consistency was noted in the detection of *Haemophilus influenzae* (Kappa = 0.104, $p < 0.001$). The two methods demonstrated general consistency in the detection of six pathogenic bacteria based on the Kappa coefficient ($0.75 > \text{Kappa} \geq 0.4$, $p < 0.001$ for all) (Table 3). The detection consistency for *Staphylococcus aureus* was poor (Kappa = 0.388, $p < 0.001$).

Based on the LAMP method, the positive detection rates of the pathogenic bacteria were as follows: 28.89% for COPD (135 cases), 32.88% for bronchiectasis (73 subjects), and 19.51% for lung cancer (41 cases). The total positive detection rate was 28.51%. The positive detection rates using the traditional culture method of the pathogenic bacteria were 25.93%, 34.25%, and 12.20% for COPD, bronchiestasis and lung cancer, respectively, whereas the average detection rate was 26.10%. However, the difference in the positive detection rates of the pathogenic bacteria between the two methods was not statistically significant (all $p > 0.05$; Table 4). A total of 9 COPD cases with LRTI (6.67%) were characterized as mixed infections by the LAMP method, whereas the culture method detected only 2 COPD cases with LRTI (1.48%) ($p < 0.05$; Table 5). The top four bacteria detected by the LAMP method in patients with COPD were *Haemophilus influenzae* (11 cases, 8.15%), Methicillin-resistant *Staphylococcus aureus* (8 cases, 5.93%), *Pseudomonas aeruginosa* (7 cases, 5.19%), and *Streptococcus pneumoniae* (7 cases, 5.19%). A total of 15 cases (11.11%) of PA infection, 3 cases (2.22%) of fungal infection, and 3 cases of *Klebsiella pneumoniae* infection (2.22%) were detected by the culture method. The detection rates of *Haemophilus influenzae* (8.15% vs 0.74%, $p = 0.003$) and MRSA (5.93% vs 0.74%, $p = 0.042$) by the LAMP method were higher than those of the culture method (Table 5). *Legionella pneumophila* could be detected by the LAMP method, but not with the culture method (Table 5). The culture method was able to detect 1 case of *Penicillium marneffei* infection (0.74%), 2 cases of *Aspergillus fumigatus* infection (1.48%), and 3 cases of *Corynebacterium striatum* infection (2.22%) compared with the LAMP method.

The pathogenic bacteria detected by the LAMP method in patients with bronchiectasis were mainly *Pseudomonas aeruginosa* (12.33%), *Streptococcus pneumoniae* (4.11%), and *Mycobacterium tuberculosis* complex (4.11%). One case of *Corynebacterium striatum* infection (1.37%) was detected by the culture

Table 6. Comparison of Pathogenic Bacteria Detected by the LAMP and Culture Methods in Patients with Bronchiectasis.

Pathogen, n (%)	The LAMP Method (n = 73)	The Culture Method (n = 73)	*p*
Streptococcus pneumoniae	3 (4.11)	4 (5.48)	> 0.999
Staphylococcus aureus	1 (1.37)	1 (1.37)	> 0.999
Methicillin-resistant Staphylococcus	2 (2.74)	0 (0.00)	---
Escherichia coli	2 (2.74)	1 (1.37)	> 0.999
Pseudomonas aeruginosa	9 (12.33)	15 (20.55)	0.180
Haemophilus influenzae	4 (2.94)	0 (0.00)	0.128
Mycobacterium tuberculosis complex / Mycobacterium	3 (4.11)	4 (5.48)	> 0.999
Corynebacterium striatum	0 (0.00)	1 (1.37)	---
Single infection	24 (32.88)	24 (32.88)	> 0.999
Mixed infections	0 (0.00)	1 (1.37)	---

Table 7. Comparison of the pathogenic bacteria detected by the LAMP and culture methods in patients with lung cancer.

Pathogen, n (%)	The LAMP Method (n = 41)	The Culture Method (n = 41)	*p*
Staphylococcus aureus	1 (2.44)	2 (4.88)	> 0.999
Methicillin-resistant Staphylococcus	2(4.88)	0 (0.00)	---
Escherichia coli	1 (2.44)	0 (0.00)	---
Klebsiella pneumoniae	1 (2.44)	0 (0.00)	---
Pseudomonas aeruginosa	1 (2.44)	1 (2.44)	> 0.999
Haemophilus influenzae	2 (4.88)	0 (0.00)	---
Candida albicans	0 (0.00)	1 (2.44)	---
Candida tropicalis	0 (0.00)	1 (2.44)	---
Single infection	8 (19.51)	5 (12.2)	0.383
Mixed infections	0 (0.00)	0 (0.00)	---

method compared with the LAMP method (Table 6). The LAMP method detected 1 cases of *Staphylococcus aureus* infection (2.44%), 2 cases of *Haemophilus influenzae* infection (4.88%), 2 case of Methicillin-resistant *Staphylococcus aureus* infection (4.88%), 1 case of *Escherichia coli* infection, and 1 case of *Klebsiella pneumoniae* infection (2.44%) in patients with lung cancer. One case of *Candida albicans* infection and 1 case of *Candida tropicalis* infection (2.44%) were detected by the culture method compared with the LAMP method (Table 7).

Discussion

In the present study, the common pathogenic bacteria noted in three pulmonary disorders combined with LRTI, notably *Haemophilus influenza*, Methicillin-resistant *Staphylococcus aureus*, and atypical pathogens were detected by the LAMP method. These results suggest that the LAMP method might play an important role in diagnosing patients with pulmonary disorders accompanied with LRTI and may provide etiological evidence to guide the use of antibiotics in clinical practice.

The LAMP method is a relatively new molecular amplification method that has the advantages of simple operation, fast and sensitive detection of a wide spectrum of pathogenic bacteria, low cost, and practical significance for the diagnosis and treatment of patients with respiratory tract infections developed in local hospitals. In general, the majority of the studies use sputum as the detection specimen. However, obtaining sputum and distinguishing pathogens from the resident bacteria in the oral cavity is difficult [22]. Bronchoalveolar lavage fluid is not readily contaminated by the normal flora of the upper respiratory tract and is not affected by external factors [23]. Given that BALF could be used to detect common pathogenic bacteria in LRTI with high sensitivity and within a limited period, the present study used BALF as the detection specimen. Moreover, the present study selected patients with specific diseases and used the results of the BALF/sputum/tissue culture method as the reference standard. The BALF/sputum/tissue was collected from the patient upon admission and sent for pathological examination. The positive detection results were analyzed with regard to the clinical manifestations associated with the presence of infectious pathogenic bacteria. Therefore, the apparent detection performance of the LAMP method could be evaluated.

Hou *et al.* demonstrated that the positive rates of pathogenic bacteria detected by the LAMP method were higher than those of the sputum culture method in sputum samples from patients with LRTI and pneumonia [7]. However, no significant differences were noted in the positive detection rates of the pathogenic bacteria present in patients with three pulmonary disorders combined with LRTI between the LAMP (based on BALF samples) and the culture methods. This result may be attributed to the patients' diseases and the different distribution of pathogenic bacteria, as well as to the lack of detection of the number of pathogenic bacteria in BALF due to sample dilution. Furthermore, based on the LAMP method applied in BALF samples from COPD patients with LRTI, it was determined that the positive rate of pathogenic bacteria was 28.89%, whereas the positive rate of pathogenic bacteria in patients with bronchiectasis combined with infections was 32.88%. Finally, the positive rate of patients with lung cancer was 19.51%.

Fastidious bacteria require specific culture conditions, resulting in a considerably difficult culture. However, the LAMP method exhibits significant advantages in the detection of fastidious bacteria, such as *Haemophilus influenzae* and *Streptococcus pneumoniae*, and can avoid such disadvantages [24]. Methicillin-resistant *Staphylococcus aureus* infection causes severe illness and is associated with increased mortality; patients with MRSA usually require hospitalization and mechanical ventilation [25]. It has been reported that the positive rate of MRSA culture in the clinic is not high [26]. In the present study, the data indicated that the LAMP method when applied to BALF could be used to successfully detect more cases of *Haemophilus influenzae*, MRSA, atypical pathogens, and mixed infections than the culture method. A clinical study has also demonstrated that atypical pathogens may not be the main pathogenic bacteria responsible for the infection of COPD in acute exacerbation [27]. Only 2 cases of *Legionella pneumophilia* were detected by LAMP in the BALF samples. Among the pathogenic bacteria, the infection or colonization of PA was an important factor in assessing the severity of bronchiectasis [28], whereas the LAMP and the culture methods demonstrated optimal consistency with regard to PA detection. In general, the culture method cannot be used to culture two types of pathogens at the same time, notably in the case of mixed infections where common bacteria are mixed with atypical pathogens, fastidious bacteria or mycobacterial species. In the present study, the LAMP method demonstrated higher sensitivity in BALF with regard to the mixed infections and could be used to detect higher number of cases of mixed infections compared with that of the culture

method. These results suggest that the LAMP method could be used to detect common pathogenic bacteria in common chronic pulmonary disorders combined with LRTI, which was possibly related to the biological characteristics of the pathogenic bacteria. In addition, it demonstrated significant advantages in the detection of fastidious bacteria, atypical pathogens, and MRSA.

However, the present study also had some limitations. The small sample size of patients with pulmonary tuberculosis leads to biased research results. Therefore, a larger sample size is required for further clinical research. Moreover, the infection of *Mycobacterium tuberculosis* is an important cause of bronchiectasis. Therefore, the possibility of *Mycobacterium tuberculosis* infection should be excluded for bronchiectasis. Due to the complexity of lung cancer and the presence of pathogenic bacteria in patients with lung cancer combined with LRTI, additional clinical research studies are required with a larger sample size to further explore the distribution of pathogenic bacteria in lung cancer. Furthermore, the present study was a single-center clinical study with a small research sample size. Sputum should be selected for the detection of pathogenic bacteria by the LAMP method and for further paired comparison. Additional clinical research is required to further explore the clinical application value of the LAMP method for chronic lung disease combined with LRTI. In addition, the etiological test was repeated as many times as possible to obtain etiological evidence. Finally, it is necessary to develop specific LAMP kits that can detect different types of pathogenic bacteria.

Conclusions

In conclusion, as compared to culture method, LAMP can detect more pathogenic bacteria, notably *Haemophilus influenza*, Methicillin-resistant *Staphylococcus aureus*, and atypical pathogens, in clinically common pulmonary disorders combined with LRTI. Loop-mediated isothermal amplification may provide etiological evidence to guide the clinical use of antibiotics in primary hospitals.

Authors' contributions

Y W and J H carried out the studies, participated in collecting data, and drafted the manuscript. Y W, Yj D and Hh Z performed the statistical analysis and participated in its design. Y W, Zs Z and Wq Z participated in the acquisition, analysis, or interpretation of data and draft the manuscript. All authors read and approved the final manuscript.

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