Comparative analysis of loop-mediated isothermal amplification combined with microfluidic chip technology and q-PCR in the detection of clinical infectious pathogens

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Abstract

Background: Rapid diagnosis of infectious pathogens at an early stage is crucial to stabilize the patient’s condition, reduce medical costs, and shorten hospital stays. Currently, some point-of-care tests have their own shortcomings. Therefore, we built a microfluidic chip based on loop-mediated isothermal amplification to can quickly and sensitively detect infectious pathogens.

Methods: We extracted the DNA of S. aureus, MRSA, Shigella and Klebsiella pneumoniae. Then, the DNA samples were diluted by 10-fold and examined by two methods: LAMP-microfluidic chip and q-PCR, the sensitivity of whom was also compared. In addition, the specificity of the two was also examined by detecting the target bacteria and other microorganisms using the same methods. Finally, we extracted and tested the DNA of clinically infected humoral samples to determine the coincidence rate between the two methods and the bacterial culture method.

Results: For S. aureus, MRSA, Shigella, and Klebsiella pneumoniae, the detection limits of the chip were 2.25×10³ copies/μl, 5.32×10³ copies/μl, 2.89×10³ copies/μl, 6.53×10² copies/μl, and the detection limits of q-PCR were 2.25×10² copies/μl, 5.32×10¹ copies/μl, 2.89×10² copies/μl, 6.53×10¹ copies/μl, respectively. In terms of detection specificity, neither method cross-reacted with other strains. For the detection of infectious humoral samples, the total coincidence rate between the q-PCR and bacterial culture method was 85.7%, 95%, 95%, and 95.5%, and the total coincidence rate between the chip and bacterial culture method was 81%, 95%, 90%, and 86.4%, respectively.

Conclusion: LAMP-microfluidic chip provides a simple, sensitive, specific, convenient, and rapid pathogen detection method for clinically infected humoral samples without relying on expensive equipment or technical personnel.

Keywords
bacteria, chip, detection, loop-mediated isothermal amplification, microfluidics
1 | INTRODUCTION

With the rampant application of antibiotics and the increasing cases of invasive operation, critically ill patients are becoming subjects with high risk of various pathogenic infections, who would suffer further worsening of the condition, prolonged hospital stay, incurred with increased treatment costs, even go through life-threatening effects since they were normally complicated with low immunity and multiple organ failure. A study based on 49,331 patients with sepsis or septic shock in 149 hospitals in New York State, United States, showed that the use of antibiotics within 3 h increased patient mortality by 1.04% for each hour delayed, and the hospitalization mortality rate for patients who took antibiotics at 3–12 h was 14% higher than patients who took antibiotics within 3 h. Therefore, timely recognition of infectious diseases, rapidly and accurately identify underlying pathogens, and then targeting effective antibiotics are key to successful treatment, not only for better prognosis but also to prevent antibiotic resistance. However, it is often difficult to accurately diagnose infectious diseases in clinical practice due to the wide variety of pathogens that cause infection, and patients may carry more than one pathogen and tend to coexist when the infection occurs. In addition, clinical and laboratory signals of severe infection, such as tachycardia, fever, and changes in white blood cell count, are nonspecific and often appear in other acute diseases. Therefore, the reasons mentioned above have rendered an accurate and rapid diagnosis of infectious diseases a formidable task. Currently, the gold standard for the diagnosis and identification of pathogenic bacteria is still bacterial culture. However, bacterial culture requires a long cycle, high requirements for inspectors, a series of bacterial identification and drug susceptibility equipment, so empiric antibiotic therapy is often required by clinicians and making bacterial resistance became an important issue affecting human society. With the rapid development of molecular biology techniques in the past decades and the emergence of the novel coronavirus 2019 (COVID-19) pandemic, point-of-care testing (POCT) has gradually become the focus of attention, especially POCT based on nucleic acid amplification. In this study, we established a POCT method based on nucleic acid amplification as an alternative method to PCR. LAMP is capable of amplifying a limited number of copies of DNA up to a million times in an hour with high specificity, efficiency, and fast under isothermal conditions. LAMP analysis involves four to six primers, including two external primers (F3 and B3), two internal primers (FIP and BIP), and loop primers (LF and/or LB). The reaction uses DNA polymerase with strand displacement function to continuously replicate and amplify DNA at a constant temperature (60–65°C), and fluorescence detection or naked-eye observation is carried out by fluorescent or colored dye incorporation method. In diagnostics based on nucleic acid amplification, replacing PCR with an LAMP reaction can eliminate the need for large volumes and expensive thermal cycling equipment, and improve the sensitivity and specificity of target gene amplification. At the same time, with the continuous development of nanomaterials, a variety of extremely sensitive biosensors have been produced for detecting markers of pathogenic microorganisms (DNA, RNA, glycoproteins, antibodies, enzymes, etc.). Biosensors for these detection devices are typically based on nanotechnological or microtechnological platforms and rely on the generation of detection signals such as optical, electrical, magnetic or visual, and when biosensors encounter a pathogen or pathogenic byproduct, they can detect a positive signal. Integrating microfluidic systems with nanomaterials and microarray technologies is highly effective for achieving the goal of miniaturization, automation, and portable chips for the analysis of complex microfluidic samples such as cells, nucleic acids and proteins. At present, there are studies that combined LAMP with microfluidic technology for the detection of nucleic acid sequences that showed a good application prospect. In this study, we combined LAMP with microfluidic chip technology, added fluorescent dyes to the constant temperature amplification reaction system, and used the fluorescent dye incorporation method for real-time fluorescence detection, and the positive sample amplification detection will produce an “S” shaped real-time fluorescence amplification curve, which completes the amplification and detection of the target gene in one step, and can simultaneously amplify a variety of nucleic acid target genes. We designed TaqMan qPCR primers and fluorescent probes to detection for single pathogens, established a detection system, and compared the differences in detection limits and cross reactions between LAMP-microfluidic chips and TaqMan qPCR methods. Furthermore, we detected humoral samples of clinical patients’ infection sites as evidence, and summarized the advantages, disadvantages, and applicability of microfluidic chips as a new detection method, so as to help the clinical practice to detect infected pathogens in a timely and accurate manner, make up for the shortcomings of traditional detection methods, and able to timely and accurately guide clinical medication.

2 | MATERIALS AND METHODS

2.1 | Instruments and reagents

This study acquired the following instruments and reagents: a VITEK MS microbial identification mass spectrometer (bioMérieux SA, Marcy L’Etine, France), a Nano-400A micro spectrophotometer (Aosheng Instrument, Hangzhou, China), a BacT/ALERT 3D automatic culture system and VITEK-2 compact ID/AST (bioMérieux SA, Marcy L’Etine, France), DNA lamp amplification reaction kits and a microfluidic chip detector (Biocare, Tianjin, China), a bacterial genome extraction kit and lysozyme (Tiangen Biotechnology, Beijing, China), a CFX96 Touch™ real-time PCR detection system (BIO-RAD, America), and a 2xGoldstar Probe Mixture (CWBIO, Beijing, China).

2.2 | Experimental strains

All bacterial strains used in the study are collected in Clinical Laboratory of PLA Rocket Characteristic Medical Center with great viability after resuscitation and confirmed by bacterial identification, drug susceptibility system and microbial identification mass spectrometer.
Standard strains: *Shigella sonnei* ATCC25931, *Acinetobacter baumannii* ATCC BAA-747, *Enterobacter cloacae* ATCC700323, *Enterococcus faecalis* ATCC29212, *Pseudomonas aeruginosa* ATCC27853, *Stenotrophomonas maltophilia* ATCC17666, *Staphylococcus aureus* ATCC29213, *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC700603, *Proteus mirabilis* ATCC35659.

External quality assessment strains: *Listeria monocytogenes*, *Shigella flexneri*, *Chryseobacterium indologenes*, *Morganella morganii*, *Enterobacter aerogenes*, *Klebsiella oxytoca*, *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus*, *Candida tropicalis*, *Candida albicans*, *C. parapsilosis*.

Clinical bacteria strains: *Listeria monocyto
gen
genes*, *Shigella flexneri*, *Chryseobacterium indologenes*, *Morganella morganii*, *Methicillin-resistant Staphylococcus aureus* (MRSA), *Carbapenem-resistant Pseudomonas aeruginosa* (CRPA), *Methicillin-resistant coagulase negative Staphylococcus* (MRCNS).

### 2.3 Clinical samples

We collected blood, urine, alveolar lavage fluid, sputum and drainage fluid and other possible samples from the patients and identified positive samples or negative samples of *S. aureus*, Klebsiella pneumoniae, MRSA and Shigella by microbiological culture.

### 2.4 DNA extraction of bacterial genomic

DNA extraction from clinical samples and bacterial strain samples was performed following the instructions of the TIANamp Bacteria DNA Kit step by step, respectively. Pretreatment of clinical samples was performed according to the kit instructions, suspend the pretreated sample 200μl media and centrifuge at 12,000rpm for 2 min, and discard the supernatant. Then, resuspend the deposit in a 200μl extracting solution containing 1 mM EDTA, 10 mM Tris–HCl, and a nucleic acid proteanct, and transfer to a cracking tube filled with glass beads, heat at 100°C for 5 min after vortex for 5 min. Then, centrifuge with a high-speed centrifuge at 12,000 rpm for 2 min, transfer the supernatant to a clean centrifuge tube, store at −20°C for later use. For the positive culture samples from lower respiratory tract secretions, 10 g/L trypsin was added to the sample and mixed well with vibration, liquefied at 37°C for 30 min. When there was no solid and stringy state, it was liquefied completely. Then, the DNA extraction was carried out and the concentration and purity of the extracted DNA are detected, and stored at −20°C for later use.

### 2.5 The design, screening, and synthesis of primers and probes

*S. aureus*, MRSA, Klebsiella pneumoniae, and Shigella primers were all synthesized using the best primers screened from prior studies. LAMP analysis of each strain involves four to six primers, including two external primers (F3 and B3), two internal primers (FIP and BIP), and loop primers (LF and/or LB), where loop primers are used to accelerate the LAMP reaction and shorten the reaction time. At the same time, in order to observe the difference between the detection capability of LAMP-microfluidic chip and real-time quantitative PCR (qPCR) methods, the primers of qPCR for the detection of a single pathogen were designed, and a detection system was established to compare the difference between the sensitivity and specificity of the microfluidic chip and qPCR. The primers of qPCR are synthesized by Sangon Biotech and shown in Table 1. The primer sequences of LAMP are not disclosed herein.

### 2.6 Real-time quantitative PCR and DNA-LAMP procedure

The LAMP detection reaction system was composed of 58μl of isothermal amplification reagent and 14.5μl of nucleic acids of the

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**Table 1** Primer sequence of TaqMan qPCR

| Species            | Target gene | Primers         | Sequence (5′–3′) |
|--------------------|-------------|-----------------|-----------------|
| *Klebsiella pneumoniae* | fimD        | Forward         | GCAGTCGGTGCCGTTTCTCT |
|                    |             | Reverse         | CGGTAATGGATACGGAAAG |
|                    |             | Probe           | ATGAGACCCGACCAACTGCA |
| *Staphylococcus aureus* | femA        | Forward         | AACGGTCAATGCGATTTAAT |
|                    |             | Reverse         | TATCTCTGCGGTTTCTTTATCAAT |
|                    |             | Probe           | TGCACTGCATAACTCAGGCAAAATGAC |
| MRSA               | mecA        | Forward         | GTGGAAGTAGATTGGGATCACGC |
|                    |             | Reverse         | TGGGCAAATTCACATTGGTTT |
|                    |             | Probe           | TCCAGGAATGCAAAAGACCAAGCATA |
| *Shigella*          | ipaH        | Forward         | CGTTCTTTGAACCACCTTTC |
|                    |             | Reverse         | GCCATGACCGGACCTGTT |
|                    |             | Probe           | CTCTGCACCGAATACCTCAGGATTCC |

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sample to be tested. Reaction parameters: 37°C, 3 min; 65°C, 50 min. 
The microfluidic chip used in this study was designed by SolidWorks 
2014 software, and injection molding of medical grade polycarbo-
nate (PC) was implemented.

The qPCR reaction consists of 10 μl of 2xGoldstar Probe Mixture 
(GoldStar Taq DNA Polymerase, PCR Buffer, dNTPs and Mg2+), 2 μl 
of DNA template, 0.4 μl (1 μM) of upstream primers, 0.4 μl (1 μM) 
of downstream primers, 0.2 μl of fluorescent probes, and 7 μl of sterile 
denatured water. The cycle was repeated 40 times at 95°C to pre-
denaturation for 10 min, 95°C for 15 s and 60°C for 30 s. The ampli-
fication was performed in the BIO-RAD CFX96TM real-time system.

To detect the presence of contamination, the same volume of 
PCR-grade deionized water was used as a negative control, where 
other conditions are the same.

2.7  |  Data analysis

A positive sample amplification test in an isothermal amplification 
reaction system produces an "S"-shaped fluorescence amplification 
curve. At the same time, the positive control test result is positive, 
and the negative control test result is negative, indicating that the test 
result is valid. If the quality control result is wrong, it is determined that 
the test result is invalid and needs to be re-examined or find the cause.

Positive sample amplification detection of qPCR reaction sys-
tem produces an "S"-shaped fluorescence amplification curve. Ct < 30 was judged positive, Ct > 35 was judged negative, and Ct val-
ues between 30 and 35 were judged to be positive after repeated 
verification.

Clinical specimen data were counted according to descriptive 
research methods, and experimental data were converted into 
a four-grid format in Table 2 and calculated according to the for-
umula. Positive coincidence rate = a/(a+c)×100%; negative coinci-
dence rate = d/(b+d)×100%; total coincidence rate = (a+d)/
(a+b+c+d)×100%.

3  |  RESULTS

3.1  |  Sensitivity

The DNA purity and concentration were measured with a Nano-
400A microspectrophotometer after bacterial culture and extrac-
tion. Then, we sequentially dilute the original DNA sample to the 
designated concentration (labeled as 10−1, 10−2, 10−3, 10−4, 10−5, 
10−6, 10−7, 10−8, 10−9, 10−10, 10−11, 10−12; the DNA concentra-
tion in tube "10−1" to "10−12" is decreased by 10 times in sequence) 
by 10 times ratio dilution method with sterile deionized water, and 
after tested using two methods, different bacterial DNA showed dif-
ferent minimum detection limits. The results are shown in Table 3 
and Figures 1–2.

3.2  |  Specificity

We extracted DNA from 36 species of bacteria after culture and 
measured their purity and concentration with a Nano-400A micro-
spectrophotometer. Then, we used the original concentration of 
DNA as a sample and used primers of S. aureus, MRSA, Shigella, and 
Klebsiella pneumoniae to, respectively, detect 36 bacteria, and the 
specificity of the two methods was shown in Table 4 and Figures 3–6.

3.3  |  Evaluation of the LAMP and q-PCR assay 
using clinical specimens

We collected more than 80 humoral samples of patients who had 
been confirmed by bacterial culture for each of the four kinds of 
clinically common bacteria to be tested and used bacterial culture as 
a reference method to assess the diagnostic value of the two detec-
tion methods. The results are shown in Tables 5 and 6.

4  |  DISCUSSION

The rapid detection system established by the combination of 
LAMP technology and microfluidic technology can extract the nu-
cleic acid of the original sample in a short period of time and de-
tect the nucleic acid simply, quickly, specifically and efficiently. This 
system amplified nucleic acid using DNA polymerase at constant 
temperature (60–65°C), saving us from complex laboratory equip-
ment. It accomplished the goal of amplifying a limited number of 
copies of DNA up to 1 million times in 1 h, and allowed simultaneous 
amplification of nucleic acids in parallel. We compared the sensitiv-
ity and specificity of the two methods using q-PCR as reference 
and summarized the practicality of the system in clinical practice.

The objective was to provide an easy, fast, and accurate way to di-
agnose bacterial species by detecting humoral specimens and guid-
ing the employment and management of antibiotics in early clinical 
stages, and to reduce mortality and alleviate the impact incurred 
on patients.

In clinical practice, the top Gram-positive bacterium in preva-
ience is S. aureus, accounting for 11% (4833/42,553). In S. aureus, 73% 
(3302/4515) of the strains are MRSA. The most commonly encoun-
tering Gram-negative bacteria are Klebsiella pneumoniae, accounting 
for 9.1% (3858/42,553), and Shigella aside from E. coli; therefore, we 
chose these five bacteria as the subjects of this study.11

| Reference method | Positive | Negative | Total |
|------------------|----------|----------|-------|
| **Experimental methods** |       |          |       |
| Positive          | a        | b        | a+b   |
| Negative          | c        | d        | c+d   |
| **Total**         | a+c      | b+d      | a+b+c+d |
In terms of detection sensitivity, existing studies have shown that both qPCR and LAMP-microfluidic chips exhibit high detection limits. In this experiment, after detecting the concentration and purity of the DNA sample, we serially diluted 12 times by 10-fold ratio dilution and labeled “10−1” to “10−12” and detected by two methods, respectively, with PCR grade sterile deionized water as a negative control. The results showed that the two methods show different detection limits for different bacteria, but the lowest detection limits of this system are lower than the q-PCR method. The detection limits of q-PCR method were S. aureus $2.25 \times 10^2$ copies/μl, Klebsiella pneumoniae $6.53 \times 10^1$ copies/μl, MRSA $5.32 \times 10^1$ copies/μl, Shigella $2.89 \times 10^2$ copies/μl. The detection limits of the microfluidic chip method were S. aureus $2.25 \times 10^3$ copies/μl, Klebsiella pneumoniae $6.53 \times 10^2$ copies/μl, MRSA $5.32 \times 10^2$ copies/μl, Shigella $2.89 \times 10^3$ copies/μl. Overall, the sensitivity of q-PCR is generally 10−102 times higher than LAMP-microfluidic chip methods. After comparing several detection methods on Alternaria solani, Khan M et al. concluded that qPCR (1.36×10^{-3} ng/μl) has the highest sensitivity and LAMP (1.36 ng/μl) has a sensitivity 10^{-3} times lower than qPCR at 63°C. This is similar to our findings. However, there are also some studies that have different conclusions from our study. Choi CW et al. showed that the sensitivity of LAMP was the same as real-time PCR after comparing the three detection methods simultaneously. In addition, it is worth mentioning that many studies have shown that the sensitivity of LAMP is generally higher than conventional PCR. The amplification results of Yersinia enterocolitica by Ranjar R et al. showed that minimum copy number of DNA detectable by LAMP and PCR assays was 44 and 440, respectively. Moosavian M et al. detected Lactobacillus pneumophila via its mip gene in respiratory specimens indicating that the detection limit of electrophoresis by PCR was <11.5 pg/μl and the results of naked-eye inspection by LAMP reaction were <1.15 pg/μl. These results suggested that detection sensitivity of LAMP was tenfold of PCR. Although studies have different views on the sensitivity of LAMP, generally, the detection limits of the two are not much different, and the minimum detection limits are very sensitive in both approaches, which back the detection of humoral specimens with low bacterial concentrations in early clinical infection.

In terms of detection specificity, the two methods showed consistent specificity, both showed solely positive signals in the target bacteria, and neither cross reacts with other bacteria. However, most reports currently suggest that LAMP testing provides higher specificity than qPCR, possibly due to the limited scope of strains covered in this study but it is certain that this is an accurate method with a wide range of detections.

A total of 83 humoral samples were collected at the Rocket Army Specialized Medical Center, including blood, urine, sputum, and drainage fluid, etc. In all, 11 humoral specimens of Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus (MRSA), Klebsiella pneumoniae, and Shigella were detected by qPCR and LAMP-microfluidic chip methods. The lowest detection limit of qPCR and LAMP microfluidic chip is shown in Table 3.

### Table 3 The lowest detection limit of qPCR and LAMP microfluidic chip

| Bacteria              | q-PCR (copies/μl) | LAMP (copies/μl) |
|-----------------------|-------------------|------------------|
| S. aureus             | $2.25 \times 10^2$ | $2.25 \times 10^3$ |
| MRSA                  | $5.32 \times 10^1$ | $5.32 \times 10^2$ |
| Shigella              | $2.89 \times 10^2$ | $2.89 \times 10^3$ |
| Klebsiella pneumonia  | $6.53 \times 10^1$ | $6.53 \times 10^2$ |

Abbreviation: MRSA, Methicillin-resistant Staphylococcus aureus.

**FIGURE 1** The dilution multiple and sensitivity result of four bacteria detected by q-PCR.
10 humoral specimens of MRSA, 12 humoral specimens of *Pneumoniae klebsiella*, and a total of 30 samples were negative for the target bacteria. In addition, we also collected 10 cases of simulated stool samples contaminated with *Shigella* and 10 cases of simulated stool samples contaminated with *Salmonella*. Both negative and positive samples had been subjected to bacterial identification and drug susceptibility testing in accordance with the operating procedures. In order to evaluate the performance of the detection system, negative and positive samples were extracted with DNA extraction kit instructions. The results are shown that for *S. aureus*, MRSA, *Shigella* and *klebsiella Pneumoniae*, the total coincidence rate between q-PCR and bacterial culture is 85.7%, 95%, 95%, 95.5% and the total coincidence rate between LAMP-microfluidic chip and bacterial culture is 81%, 95%, 90%, 86.4%, respectively. Overall, both methods have similar testing performance for humoral samples, whether in terms of positive, negative, or total coincidence rate. For samples that showed positive in bacterial culture and negative in the other two methods, we suspected that DNA loss might be due to improper manipulation during sample handling and bacterial DNA extraction. Due to the limited sample size of this study, further studies should be conducted with more sample types and sample size.

![Figure 2](image-url) The dilution multiple and positive results of four bacteria were detected by microfluidic chip method. The blue curve is the AC quality control line, and the positive signal indicates that the test is effective; TP (min) represents the jump time of the reaction curve signal.

Through the comparison of the two methods, it is not difficult to conclude that LAMP microfluidic chip has similar capabilities to qPCR in the clinical detection of infection specimens and possesses the characteristics of high sensitivity and strong detection ability, which provides a reliable basis for rapid clinical diagnosis and precise treatment. However, for ideal diagnostic tests, it should not be limited to the high sensitivity and specificity of the method. According to the requirements of the World Health Organization (WHO) for ideal diagnostic tests in developing countries, the standards for these diagnostic methods should be “Affordable, Sensitive, Specific, User-friendly (simple to perform in a few steps with minimal training), Robust and Rapid (results available in 30 min), Equipment free, and Deliverable to the end user”. So far, however, only a few rapid diagnostic tests (RDT) have met the criteria proposed by the WHO.
| Primer category | S. aureus | MRSA | Shigella | Klebsiella pneumoniae |
|-----------------|----------|------|----------|----------------------|
| No.             |          | q-PCR| LAMP     |          | q-PCR| LAMP | q-PCR| LAMP | q-PCR| LAMP |
| 1               | Enterococcus faecalis | − | − | − | − | − | − | − | − | − | − |
| 2               | Enterococcus faecium | − | − | − | − | − | − | − | − | − | − |
| 3               | MRSA | + | + | + | − | − | − | − | − | − | − |
| 4               | S. aureus | + | + | − | − | − | − | − | − | − | − |
| 5               | Staphylococcus epidermidis | − | − | + | + | − | − | − | − | − | − |
| 6               | Pseudomonas aeruginosa | − | − | − | − | − | − | − | − | − | − |
| 7               | Acinetobacter baumannii | − | − | − | − | − | − | − | − | − | − |
| 8               | CRKP | − | − | − | − | − | − | + | + | − | − |
| 9               | CRE | − | − | − | − | − | − | − | − | − | − |
| 10              | Morgan | − | − | − | − | − | − | − | − | − | − |
| 11              | Proteus mirabilis | − | − | − | − | − | − | − | − | − | − |
| 12              | Citrobacter freundii | − | − | − | − | − | − | − | − | − | − |
| 13              | Serratia marcescens | − | − | − | − | − | − | − | − | − | − |
| 14              | Stenotrophomonas maltophilia | − | − | − | − | − | − | − | − | − | − |
| 15              | Escherichia coli | − | − | − | − | − | − | − | − | − | − |
| 16              | Klebsiella pneumoniae | − | − | − | − | − | − | + | + | − | − |
| 17              | Salmonella enteritidis | − | − | − | − | − | − | − | − | − | − |
| 18              | Enterobacter cloacae | − | − | − | − | − | − | − | − | − | − |
| 19              | Chryseobacterium indologenes | − | − | − | − | − | − | − | − | − | − |
| 20              | CRPA | − | − | − | − | − | − | − | − | − | − |
| 21              | CRAB | − | − | − | − | − | − | − | − | − | − |
| 22              | Shigella sonnei | − | − | − | − | + | + | − | − | − | − |
| 23              | Yersinia enterolytica | − | − | − | − | − | − | − | − | − | − |
| 24              | Enterobacter aerogenes | − | − | − | − | − | − | − | − | − | − |
| 25              | Klebsiella oxytoca | − | − | − | − | − | − | − | − | − | − |
| 26              | Staphylococcus haemolyticus | − | − | − | − | − | − | − | − | − | − |
| 27              | Staphylococcus saprophyticus | − | − | − | − | − | − | − | − | − | − |
| 28              | S. Paratyphi A | − | − | − | − | − | − | − | − | − | − |
| 29              | Vibrio parahaemolyticus | − | − | − | − | − | − | − | − | − | − |
| 30              | Listeria monocytogenes | − | − | − | − | − | − | − | − | − | − |
| 31              | Shigella flexneri | − | − | − | − | + | + | − | − | − | − |
| 32              | Candida tropicalis | − | − | − | − | − | − | − | − | − | − |
| 33              | C. glabrata | − | − | − | − | − | − | − | − | − | − |
| 34              | Candida albicans | − | − | − | − | − | − | − | − | − | − |
| 35              | C. parapsilosis | − | − | − | − | − | − | − | − | − | − |
| 36              | Streptococcus pneumonia | − | − | − | − | − | − | − | − | − | − |
| 37              | Negative control | − | − | − | − | − | − | − | − | − | − |

Note: +, indicates positive; −, indicates negative; positive results have been marked in red.
Abbreviations: CRKP, carbapenem-resistant klebsiella pneumoniae; CRE, Carbapenem-resistant Enterobacterales; CRPA, Carbapenem-resistant Pseudomonas aeruginosa; CRAB, Carbapenem-resistant Acinetobacter baumannii; MRSA, Methicillin-resistant Staphylococcus aureus, the gene for methicillin resistance (mecA).
Currently, LAMP microfluidic chips exhibit significant advantages over many diagnostic methods based on gene amplification tests and are more likely to meet the WHO criteria for RDT. First of all, the cost of this system is low. One of the main challenges facing most diagnostic methods currently used in developing countries is the enormous cost. The main instruments required for LAMP are heating blocks or water baths, which are readily available in most laboratories, therefore, no additional charges are incurred.

A study that estimated the cost of reagents for current diagnostic techniques showed that the cost of testing a sample was the highest at $6.4–7.7 based on PCR technology, compared to just $0.71–2 for LAMP. Second, the detection speed of this method is faster. Conventional PCR reactions take about 1 h 40 min, mainly due to their long gel electrophoresis. q-PCR can usually be done within 1–2 h of the sample arriving in the laboratory. LAMP microfluidic chip can be completed within 15–60 min, which greatly saves the time spent on testing and can rapidly provide guidance for clinical diagnosis and treatment. In addition, the system is also robust. LAMP microfluidic chip has the advantage of amplifying the target DNA from partially processed or unprocessed samples. The LAMP microfluidic chip for detecting microbial nucleic acid in this study mainly includes three steps: sample preparation, amplification and detection. Sample preparation is the most important step in nucleic acid detection, especially for POC diagnosis, as it involves a complex processing procedure that often requires manual preparation, while automated processing requires large instruments in clinical laboratories. However, the method in our study from clinical specimen to sample preparation only needs to be performed in an inexpensive, small, automated microfluidic device that combines nucleic acid amplification and detection. Not only eliminates the need for DNA
CONFLICT OF INTEREST

The data; All authors wrote the manuscript and provided the final approval of manuscript. 

In short, the detection system combined with LAMP technology and microfluidic chip technology provides rapid detection of a variety of pathogens, and the system possesses several merits such as high sensitivity, strong specificity. Furthermore, the coincidence rate of clinical samples is also very high, while having the characteristics of low cost, rapidness, user-friendliness, etc., which may have considerable practical significance for the prevention and control of major diseases caused by pathogen infection.

5 | CONCLUSION

In conclusion, overall, the LAMP microfluidic chip system is a powerful clinical detection arsenal to tackle the cost and time challenge in clinical infection diagnosis and is as reliable as other existing methods based on nucleic acid amplification. Although several studies enumerated several shortcomings of LAMP microfluidic chip system and suggested further development and optimization, we still believe that this approach can be adapted to future clinical tests and provide an accurate, sensitive, faster, cheaper, and less complex detection tool for clinical bacterial infections.

AUTHOR CONTRIBUTIONS
Enqi Zhang, Hongling Ou, and Xinru Wang involved in conception and design; Lianling Jia and Wang Zhang involved in the provision of study materials or samples; Enqi Zhang, Hongling Ou, and Yemei Wang involved in the experimental technical operation; Lianling Jia, Wang Zhang, and Yemei Wang collected and assembled the data; Enqi Zhang, Hongling Ou, and Xinru Wang analyzed and interpreted the data; All authors wrote the manuscript and provided the final approval of manuscript.

CONFLICT OF INTEREST
All authors have completed the ICMJE Form for Potential Conflicts of Interest and have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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