Development and Clinical Application of a Recombinase Polymerase Amplification-Lateral Flow Strip Assay for Detection of Carbapenem-Resistant Acinetobacter baumannii

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Acinetobacter baumannii is a worldwide, primary cause of respiratory tract infections, septicemia, urinary apparatus infections, and secondary meningitis. It can be fatal. Rapid and accurate detection methods are needed to control the spread of carbapenem-resistant A. baumannii (CRAB). Current molecular diagnostic methods are limited and not suitable for on-site detection. In this study, an isothermal detection method using recombinase polymerase amplification (RPA) combined with a lateral flow strip (LFS) was developed to target the blaOXA-51 and blaOXA-23 genes of A. baumannii. The reaction was completed in about 40 min at 37°C. This method can also effectively distinguish A. baumannii and CRAB. The limit of detection of 100-101 CFU/reaction was equal to that of other detection methods. The detection accuracy was equal to that of the qPCR method with the use of clinical samples. The RPA-LFS assay is portable, rapid, and accurate and could replace existing detection methods for on-site detection of A. baumannii and CRAB.

Keywords: carbapenem-resistant Acinetobacter baumannii, recombinase polymerase amplification, lateral flow strip, blaOXA-51 gene, blaOXA-23 gene

INTRODUCTION

Acinetobacter baumannii is a common pathogen of nosocomial infections (Chung et al., 2011; Mirshekar et al., 2018). The 2020 China Antimicrobial Surveillance Network reported that number of infections caused by A. baumannii continues to increase, accounting for 17.07% of lower respiratory tract infections and a mortality rate of 35% (Shaheen et al., 2017). A. baumannii is categorized by the World Health Organization as among the most dangerous bacteria (Song et al., 2016; Tacconelli et al., 2018). In addition, A. baumannii is resistant to several antibiotics and, thus, has attracted the attention of microbiologists and doctors (Tekin et al., 2014). A. baumannii is a major cause of respiratory tract infections, septicemia, urinary apparatus infections, and secondary
meningitis (Ranjbar et al., 2020). A. baumannii is widely distributed and can survive for long periods in hospital settings, thereby posing a serious threat to patients in the intensive care unit (Perez et al., 2007). Notably, carbapenem-resistant A. baumannii (CRAB) continues to rapidly spread globally (Villegas and Hartstein, 2003).

A. baumannii is categorized into five associated subgroups based on the production of oxacillinase (OXA): OXA-51, which is intrinsic; and OXA-143, OXA-58, OXA-40, and OXA-23, which are acquired (Woodford et al., 2006; Lee et al., 2009). CARB is resistant to various antimicrobial agents, mainly due to the production of OXA and metallo-β-lactamase (Azimi et al., 2013; Ei et al., 2019). The most common CRAB isolates produce OXA-23 carbapenemase (Chen et al., 2013). The blaOXA-51 gene is an established marker for detection of A. baumannii, while the blaOXA-23 gene is the most frequent carbapenemase gene detected in CRAB isolates (Turton et al., 2006; Chen et al., 2013; Djahmi et al., 2014; Karampatakis et al., 2017).

Rapid detection of CRAB can facilitate early treatment and minimize the severity of infection. Several diagnostic methods have been reported for the detection of A. baumannii, including loop-mediated isothermal amplification (LAMP), polymerase chain reaction (PCR), quantitative PCR (qPCR), and culture-based methods (Huang et al., 2012; Wang et al., 2014; Mu et al., 2016; Nirwati et al., 2018). Although these methods have unique advantages, all are limited by time requirements, low sensitivity, need for thermocycling equipment, and dependence on trained personnel. These drawbacks may inhibit the application of these methods in the field for everyday monitoring. To combat the extensive spread of CRAB, it is important to establish an on-site diagnostic method that is simple, rapid, accurate, and inexpensive.

Recombinase polymerase amplification (RPA) is an isothermal DNA amplification technology first reported in 2006 and widely used in recent years (Piepenburg et al., 2006). The RPA system relies on recombinase (UvsX and UvsY), single-stranded binding protein (gp32), and strand-displacing DNA polymerase (Bsu) for nucleic acid amplification. The reaction is completed in about 30 min at a constant temperature of 25–42°C, usually at 37°C, (Dong et al., 2020; Wang et al., 2020; Wang et al., 2022). The amplification products of RPA can be detected using gel electrophoresis, a fluorescence detector, and a lateral flow strip (LFS) (Khater et al., 2019; Ma et al., 2021; Wang et al., 2022). However, the sensitivity of gel electrophoresis and fluorescence detection is limited outside of a laboratory. On the contrary, a LFS is suitable for simple testing and the detection results can be analyzed visually without the need for complex thermocycling equipment and trained personnel (Li et al., 2020). The RPA-LFS assay can achieve a quick response time and good accuracy when used as a diagnostic test for a variety of infectious diseases (Dong et al., 2020; Yang et al., 2021).

In this study, an accurate RPA-LFS assay for detection of CRAB was established by designing specific primers and probes for detection of the blaOXA-51 and blaOXA-23 genes. This is the first report of the detection of A. baumannii in spumut with the use of the RPA-LFS assay and to distinguish CRAB via detection of A. baumannii (blaOXA-51 without OXA-23) and A. baumannii (blaOXA-51 and OXA-23). This method can confirm infection of CRAB, but not common A. baumannii, to facilitate early treatment and prevent severe illness.

**MATERIALS AND METHODS**

**Collection of Samples and DNA Extraction**

A. baumannii and Candida albicans were obtained from the American Type Culture Collection (Manassas, VA, USA). In addition, isolates of A. baumannii (blaOXA-51 without OXA-23), isolates of A. baumannii (blaOXA-51 and OXA-23) strains, isolates of other Acinetobacter species, and isolates of common infectious pathogens were provided by The Second People’s Hospital of Lianyungang (Lianyungang, China). The spumut isolates of A. baumannii were collected from patients aged 20–50 years and hospitalised for at least one week. Swab of the wound, sputum, and urine clinical samples were obtained from the ICU hospitalized patients with clinically suspected multi-resistant infections. Information of all strains and samples are listed in Table 1. The identities of all isolates were confirmed by 16S rRNA PCR and qPCR (Huang et al., 2012; Misbah et al., 2015). All strains were cultured in Luria–Bertani broth at 37°C while shaking at 200 rpm. Cultures of 10° colony-forming units (CFU)/µL were boiled at 100°C for 10 min as DNA templates. The DNA templates were confirmed as originating from the respective pathogens by qPCR as described previously. The PCR products amplified with 16S rRNA primers were sequenced using the first generation sequencing techniques by ABI 3730XL Genetic Sequencer, and confirmed by General Biosystems Co. Ltd. (Anhui, China).

**Design of Primers and Probes**

Primers and probes for the RPA-LFS assay were designed to target the sequences of the blaOXA-51 gene (National Center for Biotechnology Information [NCBI] reference sequence: CP043953.1) and blaOXA-23 gene (NCBI reference sequence: NG_049525.1). Forward and reverse primers were designed with the Primer-Based Local Alignment Search Tool (BLAST) (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The key parameter settings were as follows: minimum and maximum product sizes, 50 and 250 bp, respectively; minimum and maximum primer sizes, 31 and 35 nt, respectively; and minimum and maximum guanine-cytosine (GC) content, 20% and 70%, respectively. Other parameters were applied at default settings. The RPA amplification products were analyzed on a 1.5% agarose gel.

The probes were designed using Primer Premier 5 software (Premier Biosoft, Palo Alto, CA, USA) based on the sequences of regions defined by the selected primer pairs. The minimum and maximum sizes of the probes were 45 and 50 bp, the minimum and maximum melting temperatures (Tm) were 50°C and 100°C, and the minimum and maximum GC contents were 20% and 80%, respectively. In addition, if the probes and primers had three consecutive matching bases, the probes were mutated to avoid false-positive results.
TABLE 1 | Bacteria strains used in this study.

| Species                                | Source                      | Strain designation | Number of samples |
|-----------------------------------------|-----------------------------|--------------------|-------------------|
| Acinetobacter baumannii                 | Reference strain            | ATCC 19606         | 1                 |
| Acinetobacter baumannii (bla<sub>OXA-51</sub> without OXA-23) | Sputum isolated strain      | #1 #2 #3 #4 #5 #6 #7 #8 #9 #10 | 10                |
| Acinetobacter baumannii (bla<sub>OXA-51</sub> and OXA-23) | Sputum isolated strain      | #1 #2 #3 #4 #5 #6 #7 #8 #9 #10 | 10                |
| Clinical samples                        | Sputum                      | N/A                | 78                |
| Clinical samples                        | Urine                       | N/A                | 49                |
| Clinical samples                        | Swab of the wound           | N/A                | 86                |
| Acinetobacter calcoaceticus             | Sputum isolated strain      | N/A                | 1                 |
| Acinetobacter lwoffi                    | Sputum isolated strain      | N/A                | 1                 |
| Acinetobacter haemolytius               | Sputum isolated strain      | N/A                | 1                 |
| Acinetobacter johnsonii                 | Sputum isolated strain      | N/A                | 1                 |
| Candida albicans                        | Reference strain            | ATCC 10231         | 1                 |
| Enterobacter cloacae                    | Sputum isolated strain      | N/A                | 1                 |
| Enterococcus faecium                    | Sputum isolated strain      | N/A                | 1                 |
| Escherichia coli O157                   | Sputum isolated strain      | N/A                | 1                 |
| Mycobacterium tuberculosis H37 Ra       | Sputum isolated strain      | N/A                | 1                 |
| Pseudomonas aeruginosa                  | Sputum isolated strain      | N/A                | 1                 |
| Staphylococcus aureus                   | Sputum isolated strain      | N/A                | 1                 |
| Staphylococcus capitis                  | Sputum isolated strain      | N/A                | 1                 |
| Staphylococcus epidermidis              | Sputum isolated strain      | N/A                | 1                 |
| Staphylococcus haemolyticus             | Sputum isolated strain      | N/A                | 1                 |
| Staphylococcus hominis                  | Sputum isolated strain      | N/A                | 1                 |
| Staphylococcus saprophyticus            | Sputum isolated strain      | N/A                | 1                 |
| Staphylococcus warneri                  | Sputum isolated strain      | N/A                | 1                 |
| Stenotrophomonas maltophilia            | Sputum isolated strain      | N/A                | 1                 |
| Streptococcus pneumonia                 | Sputum isolated strain      | N/A                | 1                 |
| Staphylococcus epidermidis              | Sputum isolated strain      | N/A                | 1                 |
| Klebsiella pneumonia                    | Sputum isolated strain      | N/A                | 1                 |

ATCC, American Type Culture Collection, Manassas, VA, USA.

RPA-LFS Procedure

RPA reactions were conducted in accordance with the manufacturer’s instructions of the Twist Amp® DNA Amplification nfo Kit (TwistDx Ltd., Maidenhead, UK). Each 25–μL reaction mixture contained 1.05 μL of each primer (10 μM), 0.3 μL of the probe (10 μM), 1.0 μL of the template, and other standard reaction components. Primers and probes were synthesized by General Biosystems Co. Ltd. To initiate the reaction, 1.25 μL of magnesium acetate (280 mM) were added. The reaction mixture was incubated for 30 min at 37°C. Then, 5 μL of the amplification product were spotted on the LFS (Ustar Biotechnologies Ltd., Hangzhou, China). The LFS was composed of a sample pad, conjugate pad (soaked with mouse-originated AuNP-tagged anti-FITC antibody), test line (coated with streptavidin), and absorbent pad that lined up through the solvent migration route. The RPA amplification product was added to the sample pad of the LFS and the stick of the LFS was inserted into 100 μL of the solvent (Ustar Biotechnologies Ltd.) for about 10 min until the test and control lines were visualized. Totally, the reaction is completed in 30 min isothermally at 37°C and the result can be observed on a LFS in 10 min.

To determine the suitability of the RPA-LFS assay to specifically detect bla<sub>OXA-51</sub> and bla<sub>OXA-23</sub>, 20 A. baumannii (bla<sub>OXA-51</sub> without OXA-23) and A. baumannii (bla<sub>OXA-51</sub> without OXA-23) isolates from sputum were used as templates (10<sup>5</sup> CFU). The specificity of the primer-probe set bla<sub>OXA-51</sub>-F3/P/R2B was tested with the RPA-LFS assay using different sample templates of microbes isolated from sputum. Reference strains of C. albicans and A. baumannii were also tested (Table 1). The amount of the templates was set at 10<sup>7</sup> CFU. The LOD was first determined with pure A. baumannii (bla<sub>OXA-51</sub> without OXA-23). The amount of template was tested at 10<sup>5</sup>–10<sup>6</sup> CFU/μL (1 μL for each reaction).

qPCR Procedure

qPCR detection procedure of A. baumannii and CRAB were performed as previously reported (Martín-Peña et al., 2013). The primers bla<sub>OXA-51</sub>-qF (5′-GCA ACC ACC ACA GAA G-3′) and bla<sub>OXA-51</sub>-qR (5′-TCC AAT ACG ACG AGC T-3′) were designed to detect A. baumannii, while the primers bla<sub>OXA-23</sub>-qF (5′-ATC GGA TTG GAG AAC C-3′) and bla<sub>OXA-23</sub>-qR (5′-CCT GAT AGA CTG GGA CT-3′) were used to detect CRAB.

RESULTS

Design of RPA Primers and Probes for CRAB Detection

The results showed that the four primer pairs produced no obvious primer-dependent artifacts when the DNA template was excluded (Figure 1A). The primer sets bla<sub>OXA-51</sub>-F2 and bla<sub>OXA-23</sub>-F2 were used to design the probes as 5′ end. Possible pairing between the probe and reverse primer of bla<sub>OXA-51</sub> was analyzed to identify the cause of false-positive signals. False-positive
signals could result if the probe and reverse primer share three or five consecutive matching bases (Figure 1B). To disrupt consecutive matching, two bases were substituted on the probe (C < G and T < G). Likewise, false-positive signals could result if the probe and reverse primer of \( \text{blaOXA-23} \) shared three or four consecutive matching bases (Figure 1C), thus five bases were substituted on the probe (A < T, A < C, T < C, A < T, and A < G).

Screening two more primers in front of probes according to the primer design principle, they were named as \( \text{blaOXA-51-F3} \) and \( \text{blaOXA-23-F3} \). The sequences of the primers and modified probes are listed in Table 2, where base substitutions are highlighted in red. The use of these two modified primer-probe sets for the RPA-LFS assay prevented false-positive signals with no DNA template control (NTC). Since the results indicate that the amplifications were not affected (Figure 1D), the modified primer-probe sets were used in this study.

**Suitability of the RPA-LFS Assay on \( \text{blaOXA-51} \) and \( \text{blaOXA-23} \)**

The use of the two primer-probe sets (\( \text{blaOXA-51-F3/P/R2B} \) and \( \text{blaOXA-23-F3/P/R2B} \)) to detect \( A. \) baumannii (\( \text{blaOXA-51} \) without \( \text{OXA-23} \)) demonstrated that the primer-probe set targeting the \( \text{blaOXA-23} \) gene did not yield a positive signal, while only the primer-probe set targeting the \( \text{blaOXA-51} \) gene obtained a positive signal and the NTC did not yield a false-positive signal (Figure 2A). Then, the primer-probe sets \( \text{blaOXA-51-F3/P/R2B} \) and \( \text{blaOXA-23-F3/P/R2B} \) were used to

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**TABLE 2** | Primers and probes used in this study.

| Primers/Probes | Primer Sequences | Size (bp) | Reaction name | Targeting area | Production Size (bp) |
|----------------|------------------|-----------|---------------|----------------|---------------------|
| \( \text{blaOXA-51-F1} \) | CTATTCCGGTTTATCAAGATTTAGCTCGTCG | 31 | RPA | 22282855..2228343 | 89 |
| \( \text{blaOXA-51-R1} \) | ATCTGCATTGCCATAACCAACACGCTGTCG | 31 | | | |
| \( \text{blaOXA-51-F2} \) | AGCTATGGTAATGATCTTGCTCGTGCTTCGA | 31 | 2228062..2222178 | 117 |
| \( \text{blaOXA-51-R2} \) | AAATACTTCTGTGGTGGTTGCCTTATGGTGC | 31 | | | |
| \( \text{blaOXA-23-F1} \) | TTTAATGGTCCTACCAACCAGAAATTATCAACCC | 35 | | | |
| \( \text{blaOXA-23-R1} \) | GTATGCGTCTTGATCTCATGCAAAAAGAAGTAAAA | 35 | 411..513 | 103 |
| \( \text{blaOXA-23-F2} \) | CGTGAACACATCTGAGGGTGGTTTCATTTATT | 35 | | | |
| \( \text{blaOXA-23-R2} \) | TTATTCGATCGTGATCTAGGCGCTTTCCTTTTCTTC | 35 | 820..910 | 91 |
| \( \text{blaOXA-51-P} \) | FITC-AGCTATGGTAATGATCTTGCTCGTCG | 47 | RPA-LFS | 2228035..2228178 | 144 |
| \( \text{blaOXA-51-R2B} \) | Biotin-AAAAATTCCTCTGTGGTGGTTCTATGGTGC | 31 | | | |
| \( \text{blaOXA-51-F3} \) | GGGATTTACAAAGGCGGATCAGGTCGTT || | |
| \( \text{blaOXA-23-P} \) | FITC-CTTGATCACTTGATCGGCGCTTTAGT || | |
| \( \text{blaOXA-23-R2B} \) | Biotin-TTTAATGGTTCTATGGTGGTTCTATGGTGC | 35 | | | |
| \( \text{blaOXA-23-F3} \) | TGCTGATCGTGATCGGCGCTTTAGATT || | |
| \( \text{blaOXA-23-R1} \) | CGTGAACACATCTGAGGGTGGTTTCATTTATT | 16 | qPCR | 2228158..2228292 | 135 |
| \( \text{blaOXA-23-R2} \) | ATCGATCGGAGAGCC | 16 | | | |

F, forward primer; R, reverse primer; P, probe.
detect the 10 A. baumannii (blaOXA-51 and OXA-23) isolates. All RPA-LFS reactions yielded positive signals without NTC. These results indicate that the primer-probe sets blaOXA-51-F3/R2B/P and blaOXA-23-F3/P/R2B can effectively distinguish strains coding for the blaOXA-51 and blaOXA-23 genes, respectively (Figure 2B). The suitability of the primer-probe sets used in this study was deemed good.

Specificity of the RPA-LFS Assay for Detection of blaOXA-51
The primer-probe set blaOXA-51-F3/P/R2B showed good specificity (Figure 3). The primer-probe set blaOXA-51-F3/P/R2B targeting the blaOXA-51 gene was highly specific and, thus, used throughout the rest of the study.

FIGURE 2 | Applicability of the primer-probe sets. (A) The image shows the detection results of the RPA-LFS assay for 10 A. baumannii (blaOXA-51 without OXA-23) isolates using the primer-probe sets blaOXA-51-F3/R2B/P (#1-1, #2-1, #3-1, #4-1, #5-1, #6-1, #7-1, #8-1, #9-1, #10-1) and blaOXA-23-F3/R2B/P (#1-2, #2-2, #3-2, #4-2, #5-2, #6-2, #7-2, #8-2, #9-2, #10-2). (B) The image shows the detection results of the RPA-LFS assay for 10 A. baumannii (blaOXA-51 and OXA-23) isolates using the primer-probe sets blaOXA-51-F3/R2B/P (#1-1, #2-1, #3-1, #4-1, #5-1, #6-1, #7-1, #8-1, #9-1, #10-1) and blaOXA-23-F3/R2B/P (#1-2, #2-2, #3-2, #4-2, #5-2, #6-2, #7-2, #8-2, #9-2, #10-2). NTC-1, no template control with the primer-probe set blaOXA-51-F3/R2B/P. NTC-2, no template control with the primer-probe set blaOXA-23-F3/R2B/P. The name of each set is shown at the top of each lane. The positions of the test and control lines are indicated on the right. All reactions were performed at 37°C for 30 min. The images represent the results of three independent experiments.

FIGURE 3 | Specificity of the primer-probe sets. The image shows the detection results of the RPA-LFS assay with different bacterial templates using the primer-probe set blaOXA-51-F3/R2B/P. The name of the bacterium used for each reaction is shown at the top of each lane. NTC, no template control. The positions of the test and control lines are indicated on the right. All reactions were performed at 37°C for 30 min. The image represents the results of three independent experiments.
Limit of Detection (LOD) of the RPA-LFS Assay for the blaOXA-51 and blaOXA-23 Genes

The results of the RPA-LFS assay showed that the LOD was $10^0$ CFU per reaction (Figure 4A). To mimic conditions of complex contamination, pure A. baumannii (blaOXA-51 without OXA-23) was spiked with $10^7$ CFU/μL of Acinetobacter lwoffi and Escherichia coli O157. In addition, $10^7$–$10^9$ CFU/μL of spiked A. baumannii (blaOXA-51 without OXA-23) were tested with the RPA-LFS assay. The results indicated that the RPA-LFS assay can tolerate interference from other bacteria and the LOD was $10^0$ CFU/μL (Figure 4B). Thus, the LOD of the RPA-LFS assay for the blaOXA-51 gene was $10^0$ CFU. Then, the LOD of the RPA-LFS assay for A. baumannii (blaOXA-51 and OXA-23) was tested using the primer-probe set blaOXA-23-F3/P/R2B against $10^7$–$10^9$ CFU/μL of A. baumannii (blaOXA-51 and OXA-23) (1 μL for each reaction). The results showed that the LOD was $10^4$ CFU per reaction (Figure 4C). In addition, the LOD of A. baumannii (blaOXA-51 and OXA-23) spiked with $10^7$ CFU/μL of A. lwoffi and E. coli O157 was also $10^7$ CFU/μL (Figure 4D).

Application of the RPA-LFS Assay for Detection of the blaOXA-51 and blaOXA-23 Genes in Clinical Samples

To mimic an actual application, the RPA-LFS assay was evaluated with 213 clinical samples. All samples were tested for the blaOXA-51 and blaOXA-23 genes with the RPA-LFS assay and compared with qPCR. The detection results of RPA-LFS were inconsistent with those of qPCR. In addition, the results showed that the detection rate of blaOXA-23 was 44.6% (Table 3).

![Image of detection results](Image)

**FIGURE 4** | LOD of the RPA-LFS assay. (A) The image shows the detection results of the RPA-LFS assay with different CFUs of A. baumannii (blaOXA-51) using the primer-probe set blaOXA-51-F3/R2B/P. (B) The image shows the detection results of the RPA-LFS assay with different CFUs of A. baumannii (blaOXA-51) and $10^7$ CFU of E. coli O157 using the primer-probe set blaOXA-51-F3/R2B/P. (C) The image shows the detection results of the RPA-LFS assay with different CFUs of A. baumannii (blaOXA-23) using the primer-probe set blaOXA-23-F3/R2B/P. (D) The image shows the detection results of the RPA-LFS assay with different CFUs of A. baumannii (blaOXA-23) and $10^7$ CFU of E. coli O157 using the primer-probe set blaOXA-23-F3/R2B/P. NTC, no template control. All reactions were performed at 37°C for 30 min. The CFUs are indicated at the top of the strips. The positions of the test and control lines are indicated on the right. The images represent the results of three independent experiments.

**TABLE 3** | Prevalence of carbapenemase genes in 213 clinical samples of A. baumannii using RPA-LFS and PCR (summarized).

| Method   | blaOXA-51 N (%) | blaOXA-23 N (%) |
|----------|----------------|-----------------|
| RPA-LFS  | 206            | 96.7            | 95              | 44.6        |
| qPCR     | 206            | 96.7            | 95              | 44.6        |
| Coincidence rate (%) | N/A         | 100%            | N/A             | 100%        |

N, number.
DISCUSSION

CRAB poses a serious threat to hospitalized patients worldwide. CRAB infections in hospitals can cause highly mortality (Tekin et al., 2014; Shaheen et al., 2017). Thus, rapid and sensitive diagnosis of CRAB in the early stage of infection is important to ensure patient safety. Current detection methods, including PCR, qPCR, and LAMP, require specific equipment that is not readily available in smaller hospitals. In addition, long periods are required to obtain the results with these methods.

Molecular detection technologies require the selection of a diagnostic amplification target for effective detection of a particular species. Many studies have evaluated various methods for detection of A. baumannii in sputum samples. With these methods, the blaOXA-51 gene is most often used as the detection target (Abhari et al., 2021). Although specific to A. baumannii, the blaOXA-51 gene cannot be used to identify CRAB. Hence, the blaOXA-23 gene has been reported as a reliable target for detection of CRAB (Tekin et al., 2014). Therefore, two primer-probe sets were designed to detect A. baumannii and CRAB.

The results of this study indicated that base modifications had no obvious effects on the LOD and the RPA-LFS assay accurately detected A. baumannii and CRAB. The LOD of the RPA-LFS assay was 10⁵ CFU for A. baumannii and 10⁴ CFU for CRAB. This sensitivity was the same as that of the real-time RPA method, which was in the range of 10⁶–10⁷ CFU per reaction (Liu et al., 2020). In addition, the RPA-LFS assay for detection of CRAB was simple and fast, as detection can be completed within 40 min (30 min for amplification and 10 min for LFS analysis). This method requires an isothermal temperature of 37°C, which can be achieved by heating with the hands. The detection results can be easily read without instrumentation in accordance with relatively simple instructions. In contrast, the PCR, qPCR, and LAMP methods require temperature control equipment and relatively long periods for the reaction. The real-time RPA method requires a shorter time than PCR, but requires the use of a fluorescence detector. The total cost of real-time RPA is higher than that of the RPA-LFS assay.

Evaluation of clinical samples showed that the accuracy of the RPA-LFS assay was good. Testing of samples from different patients showed that detection of positive samples with the RPA-LFS was equal to that of qPCR, indicating that the RPA-LFS assay presents an alternative detection method. In addition, the two LFSs used to detect A. baumannii and CRAB can be combined into one LFS in the future, which will reduce the cost.

In conclusion, the established RPA-LFS assay is simple, rapid, and accurate, does not require a laboratory facility, and can be combined with a simple and fast DNA extraction method (heat boiling) for home detection of CRAB. Timely diagnosis can facilitate early treatment of nosocomial A. baumannii infections.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

LW, GH, and XG designed the experiments and wrote the manuscript. YW, DS, and PZ collected the clinical samples. FW, DS, LC, KW, and LW performed the main experiments. YL, XL, and LW analyzed the data. All authors reviewed and approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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