Development of a LAMP method for detection of carbapenem-resistant *Acinetobacter baumannii* during a hospital outbreak

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Abstract

Introduction: Carbapenem-resistant *A. baumannii* (CRAB) represents a public health threat increasing worldwide. We assess the suitability of a loop-mediated isothermal amplification (LAMP) method for on-site screening of CRAB in a hospital facility.

Methodology: A set of six primers were designed for recognizing eight distinct sequences on six targets: *bla*<sub>OXA-23-like</sub>, *bla*<sub>OXA-24-like</sub>, *bla*<sub>OXA-51-like</sub>, *bla*<sub>OXA-58-like</sub>, *bla*<sub>IMP</sub>, and *bla*<sub>VIM</sub>. A LAMP method was developed, optimized and evaluated for the identification of CRAB in thirty-three environmental samples from an outbreak in an Intensive Care Unit (ICU) facility.

Results: The sensitivity of the LAMP assay for the detection of *A. baumannii* was ten-fold higher than the PCR assay (1.0 ng µL<sup>-1</sup>). The LAMP assays showed a higher detection rate for CRAB samples and robust diagnosis performance in comparison to a conventional PCR, with clinical sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 100% for *bla*<sub>OXA-23-like</sub>, *bla*<sub>OXA-51-like</sub> and *bla*<sub>VIM</sub>.

Conclusions: The developed LAMP assays are powerful tools that can be useful in on-site screening of CRAB causing local outbreaks in clinics and hospitals facilities where costs and equipment restraints are imperative.

Key words: *Acinetobacter baumannii*; loop mediated isothermal amplification; carbapenem resistance.

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Introduction

*Acinetobacter baumannii* is emerging as one of the most problematic pathogens in the health-care-setting, causing pneumonia, bacteremia, urinary tract infection, meningitis and wound infections [1,2]. Major risk factors for colonization or infection include exposure to an ICU, prolonged length of hospital stay, and invasive diagnostic and therapeutic procedures [3-5].

*Acinetobacter baumannii* environmental widespread and propensity to cause large and multifacility nosocomial outbreaks have been demonstrated clearly [5-7]. It can survive and persist in dry conditions for extended time periods in the patients’ environment as a result of its capacity to form biofilms on biotic and abiotic surfaces [8-10]. During outbreaks, *A. baumannii* has been recovered from various sites including furniture, patient care items and hospital equipment [9,11,12].

Furthermore, multidrug-resistant (MDR) *A. baumannii* in the hospital environment raises concerns. The emergence of carbapenem-resistant *A. baumannii* (CRAB) represents a public health threat increasing worldwide since carbapenems has been the conventional treatment for these infections few remaining therapeutic options are available [13-15]. The production of class B metallo-β-lactamases (MBLs) and class D carbapenem-hydrolysing β-lactamases (carbapenemases) have been recognized as the main mechanism of carbapenem resistance [16-19].

MBLs of particular clinical importance in *A. baumannii* are the Imipenemase (IMP) and Verona Impenemase (VIM) [20,21]. IMP-1 and VIM-1were first described in Japan in 1988 and Italy in 1999, respectively, in a strain of *Pseudomonas aeruginosa* [22,23] and its occurrence has continued to evolve [24-26].

There are four main class D OXA carbapenemases (oxacillinas) subgroups of *A. baumannii*: OXA-23-like; OXA-24-like; OXA-51-like; and OXA-58-like [14,27]. The most widespread acquired OXA gene for
carbapenem resistance in *A. baumannii* is *bla*OXA-23-like [28,29]; *bla*OXA-24-like is a chromosomal gene while *bla*OXA-58-like gene has been described as either chromosomal or plasmid-borne [30,31]. The *bla*OXA-51-like gene is typically found in *A. baumannii* and has been used in the past to identify suspected isolates of the species [32,33].

Traditional identification of *A. baumannii* in the health-care-setting has relied on classic bacterial culture, and in some cases it is guided by commercial identification systems, such as the API 20NE and Vitek 2 [7]. Nonetheless, these techniques have shown numerous disadvantages, such as time consumption, taking about three days for post identification of the species and antimicrobial susceptibility pattern. For this reason, in many cases an empirical therapy is started in patients with suspected *A. baumannii* infections until the susceptibility pattern result is obtained.

In the last decades, nucleic acid amplification tests have been developed. LAMP (Loop-mediated isothermal amplification) detects a target DNA sequence with high sensitivity and greater specificity under isothermal conditions [34-36]. It uses a strand-displacing Bst DNA polymerase, together with two internal primers (FIP, BIP) and external primers (F3, B3) that recognize six distinct sites flanking the target DNA sequences, to amplify up to 10⁹ target DNA copies [34,36]. The Loop primers (LF, LB) are additional to align the loop structures in the LAMP amplicons, accelerating the reaction and resulting in enhanced sensitivity [37]. These properties allow to significantly decrease time consumption to less than one h in isothermal conditions from 60 to 65 °C with visible product results, without expensive PCR and DNA specialized equipment [38].

Considering these advantages, LAMP may be suitable for on-site screening of CRAB causing local outbreaks in hospital facilities. This study developed LAMP detection assays for CRAB by targeting *bla*OXA-23-like, *bla*OXA-24-like, *bla*OXA-51-like, *bla*OXA-58-like, *bla*IMP and *bla*VIM genes and evaluated the assays performance with environmental samples from an outbreak in an ICU facility.

**Methodology**

**Bacterial Strains**

*Acinetobacter baumannii* reference strain AR0101 was used to design the specificity of the LAMP primers to detect the species-specific target *bla*OXA-51-like. Clinical isolates collected from Mexican hospitals [39,40]; including *A. baumannii* 9001, *A. baumannii* 8400, *A. baumannii* 7811, *P. aeruginosa* 4899 and *P. aeruginosa* 6102, known to produce OXA-23-like, OXA-24-like, OXA-58-like, IMP and VIM, respectively, were used as positive controls to determine the appropriate conditions for LAMP. Bacterial DNA extraction was performed by the phenol-chloroform method [41].

**Sample collection**

Thirty-three environmental samples were collected at a time an outbreak occurred in an ICU at a General Hospital in La Paz, Baja California Sur, México. The project was approved by Comisión Nacional de Investigación Científica del Instituto Mexicano del Seguro Social (R-2016-785-047). Samples were obtained with sterile cotton swabs, and approximately 15 cm² of each surface was swabbed. The sampling
surfaces were chosen based on the probability that they were handled by the ICU medical personnel and touched by the patient (Table 1). Subsequently, the samples were stored at -80 °C until the time of assay. The DNA extractions for all 33 swabs were conducted as described previously by removing the cotton from the swab.

**Primer design**

The LAMP assays were designed to detect the A. baumannii specific blaOXA-51-like gene (GenBank: AJ309734.2), and CRAB by targeting blaOXA-23-like (GenBank: AJ132105.1), blaOXA-24-like, (GenBank: AJ239129.2), blaOXA-58-like (GenBank: AY665723.1), blaIMP and blavIM genes. For the MBL, conserved regions were identified by clustal multiple alignments in all the latest curated β-lactamase nucleotide sequences of blaIMP and blavIM in the Bacterial Antimicrobial Resistance Reference Gene Database built by NCBI. (ftp://ftp.ncbi.nlm.nih.gov/pathogen/betalactamases/Allele.tab). For each target gene, a set of forward and reverse inner primers (FIP and BIP), forward and reverse outer primers (F3 and B3) and forward and reverse outer primers (F3 and B3)

**Table 2. LAMP primers used for assays.**

| Target      | Sequence (5′-3′) | Position    |
|-------------|-----------------|-------------|
| **blaOXA-23-like** |                |             |
| F3          | GAAGCCATGAAACGTTCCTTG | 1332-1350   |
| B3          | GTATGGTCATATGGAAGAC  | 1511-1531   |
| FIP         | ACCGAAACAAATACGCTTCCTGCAGAG | 1351-1369, 1406-1430 |
| BIP         | CTGAAATTTGACGAGGTGATTGAC-CTCTTACCTTTGAAATACG | 1435-1456, 1489-1508 |
| LF          | TTTTGCGATGAGCATAGGGGC  | 1385-1405   |
| LB          | GTGTTGATGACCATTTAAGGTT | 1465-1487   |
| **blaOXA-24-like** |                |             |
| F3          | ACTTTAGGTGAGGCAATGG | 358-376     |
| B3          | CGTTTATGTCGCAATGCT    | 551-569     |
| FIP         | TGCTATTAGCTCTAGACAGCTTCCACTG | 378-395, 418-437 |
| BIP         | GAAACACAGGATGACGTAACCTTTGACG | 478-501, 528-550 |
| LF          | CCGTCTTGAAACGTTCCTTGTT | 398-417     |
| LB          | TGGCCCTCTAAAATACCC | 507-527     |
| **blaOXA-51-like** |                |             |
| F3          | TTTGCACCTCTCCAAATGCTTA | 237-256     |
| B3          | GAGTTCAGTCTCAATAGCATC | 410-429     |
| FIP         | GCCCCGTCCTCTTAAATCT-CTCTGTACGCGGTCTTGCAG | 260-277, 300-319 |
| BIP         | AGGCTTATCCTCAGGAAACG-TGGCTCTTTTCTAATGCTG | 325-348, 388-408 |
| LF          | TGTGGTGGTTGCTCTATGG | 278-297     |
| LB          | CGATGCTATGAAAGCCTCCGCT  | 363-384     |
| **blaOXA-58-like** |                |             |
| F3          | TGGCAATATGCAAAATAGGC | 468-486     |
| B3          | CCCAGCCACTTTTAGCATC | 653-670     |
| FIP         | CCTTGGCTCAATACACCAAACT-GGAAGTTGTGAATTTTGGTGTTG | 489-511, 548-572 |
| BIP         | TTGCCTTTTAAACCTGAGATGTCCTGAGC-ATAGACGATTTCCCTCTTGCAG | 577-601, 633-652 |
| LF          | TGATATGTTGTTAATGTCAAAAGCC | 515-539 |
| LB          | TGAAGAGAGATGTTGTAAGGAGCG | 608-632     |
| **blaIMP**  |                |             |
| F3          | GCCTTTGTTCCCTAATACAGG | 196-214     |
| B3          | TTGTATATTACAGATGCATACTG | 396-417     |
| FIP         | CTCCAACAACCAAGGTACTCTTATTGTTCTTAATGCTGAGG | 221-240, 281-305 |
| BIP         | AGGCAGACATTCTCTCTCATTCT-AGATGCAATGAAACCGACTC | 323-344, 369-389 |
| LF          | CAGATCTTCTTACGCTAAAATTGAGT | 255-279     |
| LB          | ATAGGCACAGCCGGCCGCT  | 346-362     |
| **blavIM**  |                |             |
| F3          | TGTTGGCATATGCAACGAG | 190-207     |
| B3          | GCCCGAAGGACATCACG | 396-413     |
| FIP         | TCACCCACCGGCTGATCTACG-CTCCTACCCCAGCTTACGTCG | 225-243, 279-298 |
| BIP         | AACACACGCGGCGCCTTCCTCCTC-TGAAAGTGCGTGAGACGTG | 301-320, 359-377 |
| LF          | AACATACCAACCATCACGGCAATG | 245-269     |
| LB          | AAAGCAAAATTGGACTTCCCGTA | 330-351     |
reverse loop primers (LF and LB) were designed for LAMP to target eight distinct regions, using the PrimerExplorer 5.0 software (http://primerexplorer.jp/e/) (Table 2).

**LAMP reaction conditions**

Following optimization, the LAMP assays were carried out in a final volume of 25 µL and final concentrations of; 6 mM MgSO₄, 1.6 mM of dNTPs, 1 M betain (Sigma, Poole, Dorset, UK), 1X ThermoPol buffer (New England Biolabs, Hitchin, UK), 8 U of the Bst DNA polymerase large fragment (New England Biolabs, Massachusetts, USA) and genomic DNA as template. Primer sets contained 1.6 µM each of forward and reverse inner primers (FIP and BIP), 0.2 µM each of forward and reverse outer primers (F3 and B3) and 0.8 µM each of forward and reverse loop primers (LF and LB). One positive control (reference strains) and one negative control (ddH₂O) were included in each LAMP assay. Six different reaction temperatures were assessed (60, 61, 62, 63, 64, and 65 °C) and the experiments were duplicated to ensure reproducibility using a T100™ Thermal Cycler (Biorad, Hercules, USA).

**Detection of LAMP products**

The LAMP products were detected by observation of the color change according to Zhou et al. (2014) [42] by adding 1 µL of 10000X SYBR Green I at the cap of each tube before incubation to avoid further contamination. Positive amplification results were considered when a change from orange to yellowish green was observed. For further confirmation, the presence of the characteristic pattern of LAMP amplification in the positive samples was analyzed by electrophoresis in 2% agarose gels and visualized by UV transillumination.

**PCR assay**

The conventional PCR primers described previously by Brown et al. 2005 [21] were used for the intrinsic blaOXA-51-like of *A. baumannii*. The PCR amplification of CRAB was carried out using F3/B3 for each gene (Table 2). PCR comprised 35 cycles of 94 °C for one minute; 56.4 °C for one minute for blaOXA-51-like; 64 °C for blaOXA-23-like, blaOXA-24-like and blaOXA-51-like; 65 °C for blaOXA-58-like blaimp and blavim; and 72 °C for one minute in a T100™ Thermal Cycler (Biorad, Hercules, CA, USA). The PCR products were analyzed by electrophoresis in 1% agarose gel, stained with GelRed (Biotium, Hayward, USA), and visualized by UV transillumination. Each amplicon was purified with a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced (Genewiz, New Jersey, USA) using F3/B3 primers.

**Sensitivity and specificity of the LAMP assay**

The analytical sensitivity of the LAMP assay for blaOXA-51-like was compared with conventional PCR by tenfold serial dilutions of genomic DNA. *A. baumannii* AR0101 was used as a positive control, ddH₂O was used as a blank control and genomic DNA from *Salmonella* spp. was used as negative control. The analytical specificity of the LAMP assay for detecting the species-specific target blaOXA-51-like was evaluated by testing six Gram -negative bacteria (*Pseudomonas aeruginosa*, *Salmonella* spp., *E. coli* O157:H7, *Listeria monocitogenes*, and *Campylobacter jejuni*) and one Gram-positive bacteria (*Staphylococcus aureus*).

Diagnosis performance of the LAMP assays were compared with conventional PCR by the estimation of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) in a 2×2 contingency table with 95% Confidence Intervals (CI).

**Results**

**Standardization and optimization of LAMP assays**

The developed LAMP assays amplified the target sequences of blaOXA-23-like, blaOXA-24-like and blaOXA-51-like at 64 °C for 60 minutes, and blaOXA-58-like, blaIMP and blavim at 65 °C for 60 minutes (Figure 1). The
characteristic products of the LAMP reactions were analyzed upon electrophoresis (Figure 1B), which indicates the production of stem-loop DNA of the target sequence. The detection based on a color reaction with SYBR Green I showed to be a very precise method for differentiation between positive and negative tubes.

**Sensitivity of LAMP and PCR for identification of Acinetobacter baumannii**

The sensitivity of the LAMP assay and conventional PCR for detecting the species-specific target \( \text{bla}_{OXA-51}\)-like of \( A. \) baumannii were compared using ten-fold serial dilutions of genomic DNA from \( A. \) baumannii AR0101 as template (Figure 2A). The results from agarose gel electrophoresis established that the detection limit of the LAMP assay was 1.0 ng•µL\(^{-1}\) (Figure 2B), 10 times higher than that of conventional PCR (10.0 ng•µL\(^{-1}\)) (Figure 2C). To evaluate the specificity of the primers, \( \text{bla}_{OXA-51}\)-like LAMP assay was also subjected to seven different isolates without any false positive results demonstrating high specificity of the developed LAMP assay.

**Identification of CRAB in environmental samples**

Twenty of thirty-three (60.6%) environmental samples were identified as \( A. \) baumannii by amplification and sequencing of \( \text{bla}_{OXA-51}\)-like (Table 3). The remaining twenty samples were analyzed for

![Image](313x364 to 564x616)

![Image](565x726 to Image)

**Figure 2.** Sensitivity comparison of the \( \text{bla}_{OXA-51}\)-like LAMP assay and conventional PCR; (A) LAMP products detected by 1000X SYBR Green I; (B) Detection of LAMP products by agarose gel electrophoresis stained by GelRed; (C) PCR products detected by agarose gel electrophoresis stained by GelRed; Tube and lane 1: ddH\(2\)O, tube and lane 2: Salmonella sp. (negative control), tube and lane 3-9: Acinetobacter baumannii AR0101 gDNA concentrations 100.0 ng•µL\(^{-1}\), 10.0 ng•µL\(^{-1}\), 1.0 ng•µL\(^{-1}\), 100 pg•µL\(^{-1}\), 10 pg•µL\(^{-1}\), 1 pg•µL\(^{-1}\), 100 fg•µL\(^{-1}\), respectively. Lane M: 1 Kb Plus DNA Ladder in B and C.

| Sample no. | Identification | \( \text{bla}_{OXA-23}\) | \( \text{bla}_{OXA-24}\) | \( \text{bla}_{OXA-51}\) | \( \text{bla}_{OXA-58}\) | \( \text{bla}_{IMP} \) | \( \text{bla}_{AVIM} \) | \( \text{bla}_{OXA-23}\) | \( \text{bla}_{OXA-24}\) | \( \text{bla}_{OXA-51}\) | \( \text{bla}_{OXA-58}\) | \( \text{bla}_{IMP} \) | \( \text{bla}_{AVIM} \) |
|------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 2          | \( A. \) baumannii | -              | -              | +              | -              | -              | -              | -              | -              | -              | -              | -              | -              |
| 3          | \( A. \) baumannii | +              | -              | +              | -              | -              | +              | -              | -              | -              | -              | -              | -              |
| 5          | \( A. \) baumannii | -              | -              | +              | -              | -              | +              | -              | -              | -              | -              | -              | -              |
| 7          | \( A. \) baumannii | -              | -              | +              | -              | +              | +              | -              | -              | -              | -              | -              | -              |
| 11         | \( A. \) baumannii | -              | -              | +              | -              | -              | +              | -              | -              | -              | -              | -              | -              |
| 12         | \( A. \) baumannii | -              | -              | +              | -              | +              | +              | -              | -              | -              | -              | -              | -              |
| 13         | \( A. \) baumannii | -              | -              | +              | -              | +              | +              | -              | -              | -              | -              | -              | -              |
| 14         | \( A. \) baumannii | +              | -              | +              | -              | +              | +              | -              | -              | -              | -              | -              | -              |
| 15         | \( A. \) baumannii | -              | -              | +              | -              | +              | +              | -              | -              | -              | -              | -              | -              |
| 17         | \( A. \) baumannii | -              | -              | +              | -              | +              | +              | -              | -              | -              | -              | -              | -              |
| 18         | \( A. \) baumannii | -              | -              | +              | -              | -              | -              | -              | -              | -              | -              | -              | -              |
| 19         | \( A. \) baumannii | -              | -              | +              | -              | +              | +              | -              | -              | -              | -              | -              | -              |
| 21         | \( A. \) baumannii | -              | -              | +              | -              | +              | +              | -              | -              | -              | -              | -              | -              |
| 23         | \( A. \) baumannii | +              | -              | +              | -              | +              | +              | -              | -              | -              | -              | -              | -              |
| 26         | \( A. \) baumannii | -              | -              | +              | -              | +              | +              | -              | -              | -              | -              | -              | -              |
| 28         | \( A. \) baumannii | -              | -              | +              | -              | +              | +              | -              | -              | -              | -              | -              | -              |
| 29         | \( A. \) baumannii | +              | -              | +              | -              | +              | +              | -              | -              | -              | -              | -              | -              |
| 31         | \( A. \) baumannii | -              | -              | +              | -              | -              | -              | -              | -              | -              | -              | -              | -              |
| 32         | \( A. \) baumannii | +              | -              | +              | -              | +              | +              | -              | -              | -              | -              | -              | -              |
| 33         | \( A. \) baumannii | +              | -              | +              | -              | +              | +              | -              | -              | -              | -              | -              | -              |

\( ^a \) Identification by amplification and sequencing of \( \text{bla}_{OXA-51}\)-like; \( ^b \) + amplification occurred; -, amplification did not occur; \( ^c \) + amplification was observed after 60-min incubation; - amplification was not seen after 60-min incubation.
CRAB by LAMP and PCR assays. Twelve (36.4%) and six (18.2%) samples were detected to be positive for \( \text{bla}_{OXA-23}\)-like and \( \text{bla}_{VIM} \) by LAMP respectively, meanwhile six (18.2%) and five (15.1%) samples were detected by PCR. All the amplicons matched the target gene segment spanned by F3/B3 primers and shared 99-100% of identity. None of the environmental samples were positive by PCR, possessing \( \text{bla}_{OXA-23}\)-like and \( \text{bla}_{OMP} \) by LAMP and PCR.

Calculation of the clinical sensitivity, specificity, PPV, and NPV of LAMP assays yielded 100% for \( \text{bla}_{OXA-23}\)-like, \( \text{bla}_{OXA-51}\)-like and \( \text{bla}_{VIM} \), meanwhile sensitivity and NPV of PCR were 50% (CI 95%: 21.09-78.91%) and 77.78% (CI 95%: 66.53-86.04%) for \( \text{bla}_{OXA-23}\)-like, 83.33% (CI 95%: 35.88-99.58%) and 96.43% (CI 95%: 81.86-99.38%) for \( \text{bla}_{VIM} \), respectively (Table 4). The specificity and PPV was detected to be 100% for both LAMP and PCR. Overall, comparing with PCR, LAMP results highlight a robust performance of the assays.

### Discussion

The management of \( A. \text{ baumannii} \) infections represents an emerging challenge to public health worldwide [3,43]. The major threat consists in its ability to acquire antimicrobial resistance genes very quickly [44] leading to MDR and causing the appearance of strains that are resistant to almost all the existing antimicrobial agents [31,12]. Of mayor interest is the susceptibility to carbapenems since they are the last common choice for the treatment of infections caused by MDR \( A. \text{ baumannii} \), mainly through the acquisition of B and D class carbapenemases [15,43].

The propensity of \( A. \text{ baumannii} \) to cause outbreaks, promoted by MDR and tolerance to desiccation, is another important feature contributing to its perpetuation in the hospital environment [5,8]. Widespread contamination of numerous materials, furniture and hospital equipment has been found frequently, ranging from respiratory care equipment to door handles, pillows and keyboards [9,12].

This study applied the LAMP method as a visual, fast, sensitive, specific, low-cost and ready to use alternative for the detection of CRAB in inanimate hospital environments. These features were very important for on-site screening of CRAB causing local outbreaks in hospital environments. The LAMP assays were developed and optimized with six primers that recognize eight different regions on the target genes. The design of loop primers accelerated the reaction with visual results in 60 minutes by adding SYBR Green I to the tube before incubation.

LAMP had been described as a powerful amplification method with high sensitivity and low detection limits [45] and \( \text{bla}_{OXA-23}\)-like and \( \text{ bla }_{VIM} \) genes may be the most commonly acquired carbapenemase in \( A. \text{ baumannii} \) [46]. The spread of \( \text{ bla }_{OXA-23}\)-like has been established from 31% to 94% in different countries [2]. For the imipenemases IMP and VIM, results of three cohort studies conducted in Mexico from 2005 to 2012 documented imipenem resistance rates from one to 76% [42]. Therefore, our findings were congruent with other studies since the range for \( \text{bla}_{OXA-23}\)-like and \( \text{bla}_{VIM} \) genes were in the previously described limits. Although this investigation did not detect positive samples for \( \text{bla}_{OXA-23}\)-like, \( \text{bla}_{OXA-51}\)-like and \( \text{bla}_{OMP} \), these data are consistent with other reports from other countries [47], implying that \( \text{bla}_{OXA-23}\)-like and \( \text{bla}_{VIM} \) genes may be the
responsible for carbapenem resistance in these environmental samples.

Furthermore, *bla*OXA-23-like and *bla*VIM were found in tree samples closely related with hospital staff contact and handling (7: hospital bed tray, 14: mechanical ventilator touch screen and 32: telephone dial pad and handset), highlighting the importance of appropriate disinfection and rigorous measures to prevent transmission in the hospital area.

**Conclusion**

The developed LAMP assays are powerful tools for rapid detection of CRAB, which can be useful in local clinics and hospital facilities where costs and equipment limitations are imperative. The characteristics of LAMP make it possible to use near patient settings under field conditions, with one hour incubation in water baths and positive sample identification by visual observation of color change. Undoubtedly, these LAMP assays will aid in on-site screening of CRAB causing local outbreaks, potentially decreasing morbidity, nosocomial spread of *A. baumannii* and currently high levels of antibiotic resistance.

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