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Original Article

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*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-24-like}}$, *bla*$_{\text{OXA-51-like}}$, *bla*$_{\text{OXA-58-like}}$, *bla*$_{\text{IMP}}$, and *bla*$_{\text{VIM}}$. 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-1). 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*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-51-like}}$ and *bla*$_{\text{VIM}}$.

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 Imipenemase (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 (oxacillinases) 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*$_{\text{OXA-23-like}}$ [28,29]; *bla*$_{\text{OXA-24-like}}$ is a chromosomal gene while *bla*$_{\text{OXA-58-like}}$ gene has been described as either chromosomal or plasmid-borne [30,31]. The *bla*$_{\text{OXA-51-like}}$ 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.

Table 1. Locations of surface sampling performed in an Intensive Care Unit facility.

Area	Sample no.	Surface
Patient no.1	1	Mechanical ventilator buttons
	2	Hospital bed left rail
	3	Hospital bed right rail
	4	Mechanical ventilator touch screen
	5	Endotracheal tube
	6	Aspiration tube
	7	Hospital bed tray
	8	Infusion stand
	9	Hospital bed crank handle
	10	Medical trolley
Patient no.2	11	Mechanical ventilator buttons
	12	Hospital bed left rail
	13	Hospital bed right rail
	14	Mechanical ventilator touch screen
	15	Endotracheal tube
	16	Three-way stop cock
	17	Hospital bed tray
	18	Rolling hospital lamp
	19	Hospital bed crank handle
Patient no.3	20	Mechanical ventilator buttons
	21	Hospital bed left rail
	22	Hospital bed right rail
	23	Mechanical ventilator touch screen
	24	Tracheostomy tube
	25	Aspiration tube
	26	Hospital bed tray
	27	Hospital bed crank handle
	28	Window door
	29	Venturi mask
	30	Suction regulator valves
	31	Door handle
General area	32	Telephone dial pad and handset
	33	Computer keyboard

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 10^9 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*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-24-like}}$, *bla*$_{\text{OXA-51-like}}$, *bla*$_{\text{OXA-58-like}}$, *bla*$_{\text{IMP}}$ and *bla*$_{\text{VIM}}$ 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*$_{\text{OXA-51-like}}$. 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 cm^2 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 *bla*$_{\text{OXA-51-like}}$ gene (GenBank: AJ309734.2), and CRAB by targeting *bla*$_{\text{OXA-23-like}}$ (GenBank: AJ132105.1), *bla*$_{\text{OXA-24-like}}$, (GenBank: AJ239129.2), *bla*$_{\text{OXA-58-like}}$ (GenBank: AY665723.1), *bla*$_{\text{IMP}}$ and *bla*$_{\text{VIM}}$ genes. For the MBL, conserved regions were identified by clustal multiple alignments in all the latest curated β-lactamase nucleotide sequences of *bla*$_{\text{IMP}}$ and *bla*$_{\text{VIM}}$ 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

Table 2. LAMP primers used for assays.

Target	Sequence (5′–3′)	Position
bla$_{\text{OXA-23-like}}$		
F3	GAAGCCATGAAGCTTTCTG	1332-1350
B3	GTATGTGCTAATTGGGAAACA	1511-1531
FIP	ACCGAAACCAATACGTTTTACTTCT-CAGTCCCAGTCTATCAGGA	1351-1369, 1406-1430
BIP	CTGAAATTGGACAGCAGGTTGA-CTCTACCTCTTGAATAGGCG	1435-1456, 1489-1508
LF	TTTTGCATGAGATCAAGACCGA	1385-1405
LB	GGTTGGTAGGACCATTAAAGGTT	1465-1487
bla$_{\text{OXA-24-like}}$		
F3	ACTTTAGGTGAGGCAATGG	358-376
B3	CGGTTATGTGCAAGGTCAT	551-569
FIP	TGCATTAGCTCTAGGCCAGT-ATTGTCAGCAGTTCCAGT	378-395, 418-437
BIP	GGAACACAGGTCGATAATTTTTGG-CGGCAAAATTAACTTCTTGTACT	478-501, 528-550
LF	CCGTCTTGCAAGCTCTTGAT	398-417
LB	TGGCCCCCTTAAAATTACACC	507-527
bla$_{\text{OXA-51-like}}$		
F3	TTCGACCTTCAAAATGCTTA	237-256
B3	GAGTTCAAGTCCAATACGAC	410-429
FIP	GCCCGTCCCACTTAAATACT-CTTTGATCGGCCTTGAGC	260-277, 300-319
BIP	AGGCTATTCCCAGAATGGGAAAAG-AGCTAAATCTTGATAAACCGG	325-348, 388-408
LF	TGTGGTGGTTGCCTTATGGT	278-297
LB	CGATGCTATGAAAGCTTCCGCT	363-384
bla$_{\text{OXA-58-like}}$		
F3	TGGCAATATGCAAATAGGC	468-486
B3	CCCAGCCACTTTTAGCAT	653-670
FIP	CCTTGGGCTAAATCATACACAAACT-GGAAGTTGATCAATTTTGGTTGA	489-511, 548-572
BIP	TTGCCTTTTAAACCTGAAGTTCAGC-ATAGACGATTCTCCCCTCTG	577-601, 633-652
LF	TGTATAGGTGTAATTGTCAAAGGCC	515-539
LB	TGAAAGAGATGTTGTATGTAGAGCG	608-632
bla$_{\text{IMP}}$		
F3	GCGTTGTTCCTAAACATGG	196-214
B3	TTGTTAATTCAGATGCATACGT	396-417
FIP	CTCCACAAACCAAGTGACTAACTTT-GGTTCTTGTAAATGCTGAGG	221-240, 281-305
BIP	AGGCAGCATTTCCTCTCATTTT-AGATCGAGAATTAAGCCACTC	323-344, 369-389
LF	CAGTATCTTTAGCCGTAAATGGAGT	255-279
LB	ATAGCGACAGCACGGGC	346-362
bla$_{\text{VIM}}$		
F3	TGGTCGCATATCGCAACG	190-207
B3	GCCCGAAGGACATCAACG	396-413
FIP	TCGCACCCCACGCTGTATCA-GGTCTACCCGTCCAATGGT	225-243, 279-298
BIP	AACACAGCGGCACTTCTCGC-TGAAAGTGCGTGGAGACTG	301-320, 359-377
LF	AACTCATCACCATCACGGACAATGA	245-269
LB	AAAGCAAATTGGACTTCCCGTA	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).

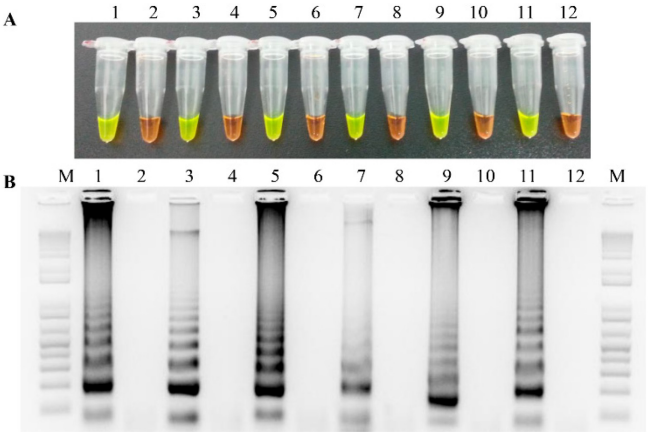
LAMP reaction conditions

Following optimization, the LAMP assays were carried out in a final volume of 25 µL and final concentrations of; 6 mM $MgSO_4$, 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.

Figure 1. Visualization of the LAMP assays. (A) LAMP products detected by 1000X SYBR Green I; (B) Detection of LAMP products by agarose gel electrophoresis stained by GelRed. Tube and lane 1: Acinetobacter baumannii AR0056 gDNA (blaOXA-23-like), 3: A. baumannii AR0088 gDNA (blaOXA-24-like), 5: A. baumannii AR0101 gDNA (blaOXA-51-like), 7: A. baumannii AR0063 gDNA (blaOXA-58-like), 9: Pseudomona aeruginosa AR0230 gDNA (blaVIM), 11: P. aeruginosa AR0095 gDNA (blaIMP). Tubes and lanes 2,4,6,8,10 and 12: ddH2O. Lane M: 1 Kb Plus DNA Ladder in B.



PCR assay

The conventional PCR primers described previously by Brown *et al.* 2005 [21] were used for the intrinsic $bla_{\text{OXA-51-like}}$ 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 $bla_{\text{OXA-51-like}}$; 64 °C for $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-51-like}}$; 65 °C for $bla_{\text{OXA-58-like}}$ bla_{IMP} and bla_{VIM}; 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 $bla_{\text{OXA-51-like}}$ 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 $bla_{\text{OXA-51-like}}$ 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 $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-51-like}}$ at 64 °C for 60 minutes, and $bla_{\text{OXA-58-like}}$, bla_{IMP} and bla_{VIM} 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 *bla*$_{\text{OXA-51-like}}$ 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, *bla*$_{\text{OXA-51-like}}$ 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 *bla*$_{\text{OXA-51-like}}$ (Table 3). The remaining twenty samples were analyzed for

Figure 2. Sensitivity comparison of the blaOXA-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: ddH2O, 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.

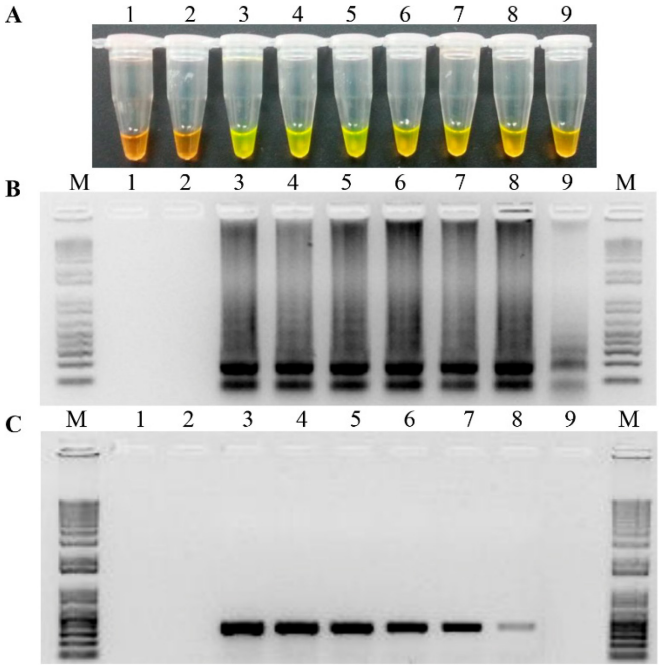


Table 3. Carbapenem-resistant *Acinetobacter baumannii* detection in environmental samples by PCR and LAMP methods.

Sample no.	Identification[a]	PCR[b]						LAMP[c]					
		bla$_{\text{OXA-23-like}}$	*bla*$_{\text{OXA-24-like}}$	*bla*$_{\text{OXA-51-like}}$	*bla*$_{\text{OXA-58-like}}$	*bla*$_{\text{IMP}}$	*bla*$_{\text{VIM}}$	*bla*$_{\text{OXA-23-like}}$	*bla*$_{\text{OXA-24-like}}$	*bla*$_{\text{OXA-51-like}}$	*bla*$_{\text{OXA-58-like}}$	*bla*$_{\text{IMP}}$	*bla*$_{\text{VIM}}$
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 *bla*$_{\text{OXA-51-like}}$; [b] + amplification occurred; -, amplification did not occur; [c] + amplification was observed after 60-min incubation; - amplification was not seen after 60-min incubation.

Table 4. Evaluation of LAMP and PCR methods for the detection of CRAB in environmental samples.

	No. of samples				LAMP VS PCR							
	LAMP		PCR		Sensitivity (%)	CI (%)	Specificity (%)	CI (%)	PPV (%)	CI (%)	NPV (%)	CI (%)
	Positive	Negative	Positive	Negative								
bla$_{\text{OXA-23-like}}$	12	21	6	27	100/50	73.54- 100/21.09-78.91	100/100	83.89- 100/ 83.89-100	100/100	-/-	100/ 77.78	-/ 66.53-86.04
bla$_{\text{OXA-51-like}}$	20	13	20	13	100/100	83.16-100/83.16-100	100/100	75.29-100/75.29-100	100/100	-/-	100/100	-/-
bla$_{\text{VIM}}$	6	27	5	28	100/83.33	54.07-100/35.88-99.58	100/100	87.23-100/87.23-100	100/100	-/-	100/96.43	-/81.86-99.38

PPV: Positive predictive value; NPV: Negative predictive value; CI: Confidence intervals (95%); -: not applicable.

CRAB by LAMP and PCR assays. Twelve (36.4%) and six (18.2%) samples were detected to be positive for *bla*$_{\text{OXA-23-like}}$ and *bla*$_{\text{VIM}}$ 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 tested positively for *bla*$_{\text{OXA-24-like,}}$ *bla*$_{\text{OXA-58-like}}$ and *bla*$_{\text{IMP}}$ by LAMP and PCR.

Calculation of the clinical sensitivity, specificity, PPV, and NPV of LAMP assays yielded 100% for *bla*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-51-like}}$ and *bla*$_{\text{VIM}}$, meanwhile sensitivity and NPV of PCR were 50% (CI 95%: 21.09-78.91%) and 77.78% (CI 95%: 66.53-86.04%) for *bla*$_{\text{OXA-23-like}}$, 83.33% (CI 95%: 35.88-99.58%) and 96.43% (CI 95%: 81.86-99.38%) for *bla*$_{\text{VIM}}$, 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. baumanii* 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. baumannii*, mainly through the acquisition of B and D class carbapenemases [15,43].

The propensity of *A. 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]. The developed LAMP assay for detecting *A. baumannii* was 10-fold higher sensitive than conventional PCR assay. Comparable results were found in previous studies for detection of *Streptococcus pneumoniae*, *Listeria monocytogenes* and *Staphylococcus* strains among many other pathogens where LAMP assay efficiency was 10- to 1,000-fold greater than that of PCR [45,46].

The CRAB assays performed on the thirty-three environmental samples showed positive results in 12/33 (36.4%) and 6/33 (18.2%) samples detected by LAMP and 6/33 (18.2%) and 5/33 (15.1%) samples detected by PCR, possessing *bla*$_{\text{OXA-23-like}}$ and *bla*$_{\text{VIM}}$ genes, respectively. Thus, compared to the conventional PCR the lower detection limit of the LAMP assays yielded a higher detection rate for CRAB samples and showed a higher diagnosis-based performance with no false positive or false negative observed.

As described previously, the *bla*$_{\text{OXA-23-like}}$ gene was the most commonly acquired carbapenemase in *A. baumannii* [46]. The spread of *bla*$_{\text{OXA-23-like}}$ 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 *bla*$_{\text{OXA-23-like}}$ and *bla*$_{\text{VIM}}$ were in the previously described limits. Although this investigation did not detect positive samples for *bla*$_{\text{OXA-24-like}}$, *bla*$_{\text{OXA-58-like}}$ and *bla*$_{\text{IMP}}$, these data are consistent with other reports from other countries [47], implying that *bla*$_{\text{OXA-23-like}}$ and *bla*$_{\text{VIM}}$ genes may be the

responsible for carbapenem resistance in these environmental samples.

Furthermore, $bla_{\text{OXA-23-like}}$ 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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