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Original Article

Antibacterial effect of acetic acid during an outbreak of carbapenem-resistant *Acinetobacter baumannii* in an ICU (II)

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Abstract

Introduction: Acetic acid (AA) has been commonly used in medicine as an antiseptic agent for the past 6000 years. This study evaluated the antibacterial effect of AA during an outbreak in an intensive care unit (ICU) facility in Baja California Sur, México.

Methodology: Thirty-five environmental samples were collected, subsequently, disinfection with AA (4%) was performed, and two days later the same areas were sampled inside the ICU facility. Carbapenem-resistant *A. baumannii* (CRAB) was detected with loop-mediated isothermal amplification assay (Garciglia-Mercado et al. companion paper), 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. CRAB isolates before and after disinfection were compared by PFGE.

Results: Eighteen (54.5%) and five (14.3%) of thirty-five environmental samples were identified as *Acinetobacter baumannii* before and after disinfection, respectively, showing a significant decrease of 85.7% ($p < 0.05$) both by Loop-mediated isothermal amplification (LAMP) and polymerase chain reaction (PCR). Furthermore, the presence of *bla*$_{\text{OXA-23-like}}$ and *bla*$_{\text{OXA-58-like}}$ genes significantly decreased ($p < 0.05$) both by LAMP and PCR methods. PFGE genotype showed high similarity among CRAB isolates before and after disinfection, suggesting wide clonal dissemination in the ICU facility.

Conclusions: This study demonstrated the novel application of AA with the LAMP assays developed for detecting CRAB. AA promises to be a cheap and efficacious disinfectant alternative to both developed and especially developing countries, preventing the spread of this organism in the environment and to other susceptible patients in health care settings.

Key words: Acetic acid; Carbapenem-resistance; *Acinetobacter baumannii*; loop-mediated isothermal amplification.

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Introduction

Acinetobacter baumannii represents a serious public health concern due to its ability to cause multifacility nosocomial outbreaks [1,2]. It has shown to survive in hospital environments for extended periods as a result of its capacity to form sessile biofilms found in various sites, including furniture, patient care items and hospital equipment [3,4]. Since carbapenems are the common choice to treat infections caused by this pathogen, the emergence of Carbapenem-resistant *A. baumannii* (CRAB) is leaving few remaining therapeutic options [5]. The production of class B metallo-β-lactamases (MBLs) and class D carbapenem-hydrolyzing β-lactamases (carbapenemases) have been recognized as the main mechanism of carbapenem resistance [6,7]. MBLs of particular clinical importance in *A. baumannii* are the Imipenemase (IMP) and Verona Imipenemase (VIM) [8]. Four main class D OXA carbapenemases (oxacillinases) subgroups of *A. baumannii* have been identified as OXA-23-like; OXA-24-like; OXA-51-like; and OXA-58-like [9]. Due to the reducing efficacy of antibiotics against CRAB, high doses of biocides are required to treat these organisms growing in fomites,

but they do not prevent bacterial colonization and biofilm formation. Therefore, interest has grown in reevaluating the novel applications of 'household remedies' as salvage procedures. Acetic acid (AA) or vinegar has been commonly used in medicine as an antiseptic agent for the past 6000 years [10], showing excellent bactericidal effect in the treatment of the plague, war wounds, and ear, chest and urinary tract infections [11,12]. It has also been used in eliminating bacteria in beef carcass [13] and fresh products [14]. Although clinical studies and observations provide evidence in support of the clinical utility of AA as an antiseptic [15], recent literature is scarce on its use as a general disinfectant. Considering this potential application, the objective of this study was to evaluate the antibacterial effect of AA during an outbreak in an intensive care unit (ICU) facility; CRAB was detected in environmental samples with the developed loop-mediated isothermal amplification (LAMP) assays (Garciglia-Mercado et al. companion paper), targeting $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like,}}$ $bla_{\text{OXA-58-like}}$, bla_{IMP} and bla_{VIM} genes.

Methodology

Bacterial Strains and DNA extraction

Reference strains used as positive controls are addressed in our companion paper (Garciglia-Mercado et al). CRAB DNA extraction was performed by the phenol-chloroform method [16].

Sample collection and disinfection

First sample collection

Prior to disinfection, thirty-five environmental samples were collected from an outbreak in an ICU at Hospital General de Zona No. 1 Instituto Mexicano del Seguro Social (IMSS) in La Paz, Baja California Sur, México. The project was approved by the Comisión Nacional de Investigación Científica del Instituto Mexicano del Seguro Social (R-2016-785-047). The surfaces of 3-bed spaces and a general area were sampled. Patients 1 and 2 were females with diagnosed pneumonia and patient 3 was a male with bacteremia in the isolation room (Table 1). Samples were obtained with sterile cotton swabs, and approximately 15 cm^2 of each surface was swabbed (see Garciglia-Mercado *et al.* companion paper for additional details of the sampling efforts). After sampling, the swabs were immediately placed in Luria-Bertani (LB) media in 2-mL tubes and incubated at 35 °C for 24 h. After incubation in the enrichment broth, the same swab was inoculated onto selective MacConkey agar plates and rotated completely. Plates were streaked for isolation and incubated at 37°C for 24 h. Light-pink or purplish colonies were selected for DNA extraction.

Disinfection

After the first sample collection, hospital staff performed the disinfection in the ICU using 4% (v/v) AA with clean wipes; the contact time was 10 min. All the areas were disinfected with the exception of the mechanical ventilators touch screens (Table 1).

Second sample collection

Two days after disinfection, the same areas were sampled inside the ICU facility (Table 1), and the swabs were processed as described previously.

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
34	Main hall floor
35	Patient no. 3 floor

Loop-mediated isothermal amplification reaction conditions

The LAMP assays were performed in a total of 25 µl reaction as previously described (Garciglia-Mercado *et al*. companion paper). For the detection of $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-51-like}}$ the tubes were incubated at 64 °C for 60 min and $bla_{\text{OXA-58-like}}$, bla_{IMP} and bla_{VIM} at 65 °C for 60 min. One positive (reference strains) and one negative (ddH_2O) controls were included in each LAMP run, and the experiments were performed in duplicate to ensure reproducibility using a T100™ Thermal Cycler (Biorad Hercules, CA, USA).

Detection of Loop-mediated isothermal amplification products

The LAMP products were detected by color change by staining with 10000X SYBR Green I (Sigma, Poole, Dorset, UK) as previously described [17]; Garciglia-Mercado *et al*. companion paper]. 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 ultra-violet (UV) transillumination.

Polymerase chain reaction assay

The conventional PCR primers described previously by Brown *et al.* (2005) [18] were used for the intrinsic $bla_{\text{OXA-51-like}}$ of *A. baumannii*. The PCR amplification of CRAB was performed by using F3/B3 for each gene; see Garciglia-Mercado *et al*. companion paper for additional details of amplification and primers used in the assays.

Molecular typing

The CRAB isolates from the environmental samples before and after disinfection, were also analyzed by Pulsed-field gel electrophoresis (PFGE) performed according to Kaufmann's method [22]. DNA was digested with *ApaI* enzyme, separated in 1% agarose gel and stained with ethidium bromide. The macrorestriction patterns were analyzed using the Tenover criteria [23] and compared by the Dice similarity index, with band tolerance and optimization set at 1.5%, using BioNumerics software (Applied Maths, Belgium). Isolates exhibiting at least 80% similarity were clustered into a pulsotype.

Statistical analysis

Diagnosis performance of the LAMP assays were compared with conventional PCR by estimating

Table 2. Carbapenem-resistance *Acinetobacter baumannii* detection by PCR and LAMP methods before and after disinfection with acetic acid (4%).

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_{VIM}	bla_{IMP}	$bla_{\text{OXA-23-like}}$	$bla_{\text{OXA-24-like}}$	$bla_{\text{OXA-51-like}}$	$bla_{\text{OXA-58-like}}$	bla_{VIM}	bla_{IMP}
Before disinfection													
1	*A. baumannii*	+	-	+	-	-	-	+	+	+	-	-	-
2	*A. baumannii*	-	-	+	-	-	-	-	-	+	-	-	-
4	*A. baumannii*	+	-	+	+	-	-	+	-	+	+	-	-
5	*A. baumannii*	+	-	+	-	-	-	+	-	+	-	-	-
6	*A. baumannii*	+	-	+	-	-	-	+	-	+	-	-	-
7	*A. baumannii*	+	-	+	-	-	-	+	-	+	-	-	-
9	*A. baumannii*	+	-	+	-	-	-	+	-	+	-	-	-
10	*A. baumannii*	+	-	+	-	-	-	+	-	+	-	-	-
13	*A. baumannii*	+	-	+	+	-	-	+	+	+	+	-	-
14	*A. baumannii*	+	-	+	-	-	-	+	-	+	-	-	-
15	*A. baumannii*	+	-	+	-	-	-	+	+	+	-	-	-
23	*A. baumannii*	+	+	+	+	-	-	+	+	+	+	-	-
25	*A. baumannii*	+	+	+	-	-	-	+	+	+	-	-	-
26	*A. baumannii*	+	-	+	+	-	-	+	-	+	+	-	-
27	*A. baumannii*	+	-	+	-	-	-	+	-	+	-	-	-
32	*A. baumannii*	+	-	+	-	-	-	+	+	+	+	-	-
34	*A. baumannii*	-	+	+	+	-	-	-	+	+	+	-	-
35	*A. baumannii*	+	-	+	-	-	-	+	-	+	-	-	-
After disinfection													
4	*A. baumannii*	-	-	+	-	+	-	+	+	+	-	+	-
11	*A. baumannii*	+	-	+	-	+	-	+	+	+	-	+	+
23	*A. baumannii*	-	-	+	-	-	-	-	-	+	-	-	-
26	*A. baumannii*	-	+	+	-	-	-	-	+	+	-	-	-
32	*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 observed after 60-min incubation.

sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) in a 2×2 contingency table with 95% Confidence Intervals (CI). The difference between the first sample collection (before disinfection) and the second sample collection (after disinfection) were determined with Chi-square test with a significance set at $p < 0.05$, using Minitab® statistical software (Minitab 18, Minitab Inc. State College, PA, USA).

Results

Identification of Carbapenem-resistant Acinetobacter baumannii

Before disinfection with Acetic Acid

Eighteen of thirty-five (54.5%) environmental samples were found to contain *A. baumannii* by amplification and sequencing of *bla*$_{\text{OXA-51-like}}$. These samples were analyzed for CRAB by LAMP and PCR assays. Sixteen (45.71%), seven (21.2%) and six (17.1%) samples were detected to be positive by LAMP, where sixteen (45.71%), three (8.6%) and five (14.3%) samples were detected by PCR, for *bla*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-24-like}}$ and *bla*$_{\text{OXA-58-like}}$ respectively (Table 2). All the amplicons matched the target gene segment spanned by F3/B3 primers and shared 99-100% of identity. None of the samples tested positively for *bla*$_{\text{IMP}}$ and *bla*$_{\text{VIM}}$ by LAMP and PCR. Calculation of the clinical sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of LAMP assays yielded 100% for *bla*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-24-like}}$, *bla*$_{\text{OXA-51-like}}$, *bla*$_{\text{OXA-58-like}}$ (Table 3), while sensitivity and NPV of PCR were 42.86% (CI95%: 9.90-81.59%) and 87.50% (CI95%: 78.66-93.01%) for *bla*$_{\text{OXA-24-like}}$, 83.33% (CI95%: 35.88-99.58%) and 96.67% (CI95%: 82.89-99.43%) for *bla*$_{\text{OXA-58-like}}$, respectively (Table 3).

After disinfection with Acetic Acid

After disinfection with AA five of thirty-five (14.3%) environmental samples were identified as *A. baumannii* by amplification and sequencing of *bla*$_{\text{OXA-51-like}}$, showing a significant decrease ($P < 0.05$) both by LAMP and PCR of its presence in the ICU facility. These samples were analyzed for CRAB by LAMP and PCR assays. Three (8.6%) samples were detected to be positive for *bla*$_{\text{OXA-23-like,}}$ *bla*$_{\text{OXA-24-like}}$, *bla*$_{\text{VIM}}$, and one (2.9%) for *bla*$_{\text{IMP}}$ by LAMP, respectively, where two (5.7%), one (2.9%), two (5.7%) and 0 (0%) samples were detected by PCR (Table 2). The presence of *bla*$_{\text{OXA-23-like}}$ and *bla*$_{\text{OXA-58-like}}$ genes significantly decreased ($p < 0.05$) after disinfecting both by LAMP and PCR methods (Table 3). Clinical sensitivity, specificity, PPV, and NPV of LAMP assays yielded 100% for *bla*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-24-like}}$, *bla*$_{\text{OXA-51-like}}$, *bla*$_{\text{VIM}}$ and *bla*$_{\text{IMP}}$ after disinfection. Sensitivity and NPV of PCR were 33.33% (CI95%: 0.84-90.57%) and 94.12% (CI95%: 87.79-97.27%) for *bla*$_{\text{OXA-24-like}}$, 66.67% (CI95%: 9.43-99.16%) and 96.97% (CI95%: 86.59-99.37%) for *bla*$_{\text{OXA-23-like}}$ and *bla*$_{\text{VIM}}$, respectively. The specificity and PPV was detected to be 100% for both LAMP and PCR. Overall, comparing with PCR, LAMP results highlighted a robust performance of the assays.

Table 3. Evaluation of LAMP and PCR methods for the detection of CRAB in an ICU before and after disinfection with acetic acid (4%).

	No. of samples				LAMP VS PCR							
	LAMP		PCR		Sensitivity (%)	CI (%)	Specificity (%)	CI (%)	PPV (%)	CI (%)	NPV (%)	CI (%)
	Positive	Negative	Positive	Negative								
Before disinfection												
bla$_{\text{OXA-23-like}}$	16	19	16	19	100/100	79.41-100/79.41-100	100/100	82.35-100/82.35-100	100/100	-/-	100/100	-/-
bla$_{\text{OXA-24-like}}$	7	28	3	32	100/42.86	59.04-100/9.90-81.59	100/100	87.66-100/87.66-100	100/100	-/-	100/87.50	-/78.66-93.01
bla$_{\text{OXA-51-like}}$	18	17	18	17	100/100	81.47-100/81.47-100	100/100	80.49-100/80.49-100	100/100	-/-	100/100	-/-
bla$_{\text{OXA-58-like}}$	6	29	5	30	100/83.33	54.07-100/35.88-99.58	100/100	88.06-100/88.06-100	100/100	-/-	100/96.67	-/82.89-99.43
bla$_{\text{VIM}}$	0	35	0	35	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
bla$_{\text{IMP}}$	0	35	0	35	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
After disinfection												
bla$_{\text{OXA-23-like}}$	3[a]	32	2[a]	33	100/ 66.67	29.24-100/9.43-99.16	100/100	89.11-100/ 89.11-100	100/100	-/-	100/ 96.97	-/ 86.59-99.37
bla$_{\text{OXA-24-like}}$	3	32	1	34	100/33.33	29.24-100/0.84-90.57	100/100	89.11-100/89.11-100	100/100	-/-	100/94.12	-/87.79-97.27
bla$_{\text{OXA-51-like}}$	5[a]	30	5[a]	30	100/100	47.82-100/47.82-100	100/100	88.43-100/88.43-100	100/100	-/-	100/100	-/-
bla$_{\text{OXA-58-like}}$	0[a]	35	0[a]	35	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
bla$_{\text{VIM}}$	3	32	2	33	100/66.67	29.24-100/9.43-99.16	100/100	89.11-100/89.11-100	100/100	-/-	100/96.97	-/86.59-99.37
bla$_{\text{IMP}}$	1	34	0	35	100/0	2.50-100/0.00-97.50	100/100	89.72-100/89.72-100	-/-	-/-	100/97.14	-/97.14-97.14

PPV: Positive predictive value; NPV: Negative predictive value; CI: Confidence intervals (95%); -: not applicable; [a]: Statistical difference ($P < 0.05$), n = 35.

Molecular typing

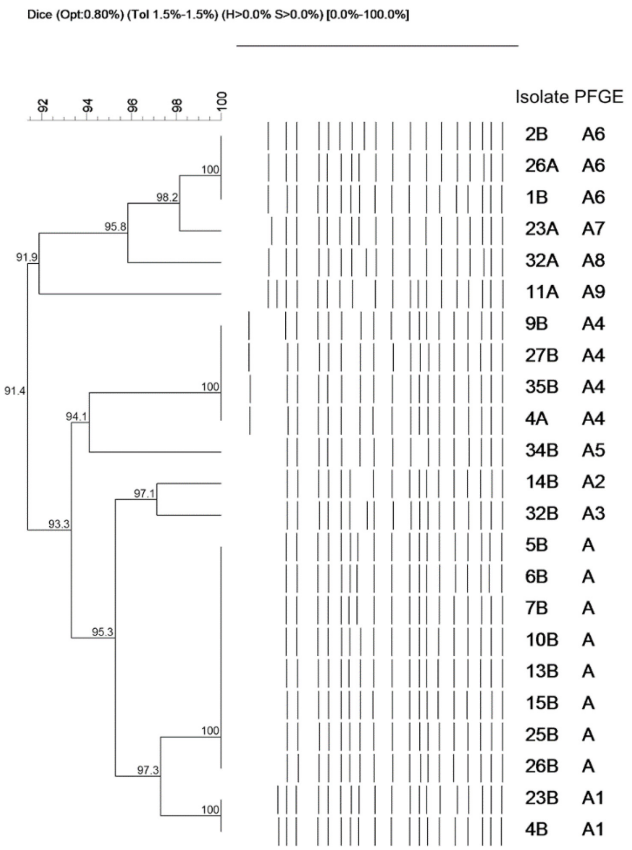
PFGE fingerprint analysis was performed in the 23 CRAB environmental isolates. A major clone A with 9 subtypes (A to A9) was identified, including all the 23 isolates from before and after disinfection (Figure 1). The subtype variants showed similar values that were always higher than 91%.

Discussion

Earlier studies have reported the use of AA as a topical agent for the treatment of burn infections and skin and soft tissue infections [19]. Planktonic growth prevention and biofilm eradication has generally been reported in problematic Gram-negative but also in Gram-positive bacteria, with concentrations ranging from 0.31% to 5% [10,12]. Moreover, the authors found excellent antibacterial effect against multi-drug resistant organisms, such as *Pseudomonas aeruginosa* and *A. baumannii* [10,15].

The antibacterial effect of AA has been thought to be due to a variety of mechanisms, of which a physical alteration of the bacterial cell wall is the most accepted theory. Since weak acids can cross bacterial membranes more easily than strong ones, the internalized AA will dissociate, acidifying the cytoplasm, which can cause acid-induced protein unfolding, membrane, and DNA damage [12]. This effect could explain the mayor susceptibility of Gram-negative compared to Gram-positive bacteria, caused by the lack of murein layer.

Figure 1. PFGE comparison of CRAB isolates before (B) and after (A) disinfection with acetic acid (4%). Letter B or A was assigned next to the corresponding sample number (Table 1). Similarity (%) among patterns is represented by the numbers beside the nodes.



With the ever-limited antibiotic and disinfectant choices for multi-drug resistant strains as CRAB, AA is a potential alternative to traditional antimicrobials (e.g. chlorine and cefotaxime) for preventing colonization and biofilm formation in hospital environments. This study found that in the disinfected areas with AA, *A. baumannii* showed a decrease of 85.7% ($p < 0.05$).

Interestingly, PFGE genotype showed high similarity among CRAB isolates before and after disinfection, suggesting wide clonal dissemination in the ICU facility (Fig. 1) where transmission through contaminated hands of healthcare staff and equipment is likely to be the main route for the spread of the microorganisms.

Furthermore, the presence of the target genes for carbapenem resistance was diminished after disinfection, showing a decrease in $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and an elimination of $bla_{\text{OXA-58-like}}$ in the environmental samples. However the presence of bla_{VIM} and bla_{IMP} increased in the same areas that were not disinfected (mechanical ventilators touch screens and buttons, and the telephone dial pad and handset from staff), confirming that these organisms acquire carbapenem resistance under antimicrobial selective pressure [20,21]. Finally, the validation of the LAMP assays with the PCR method showed a higher detection rate for CRAB samples and a higher diagnosis-based performance with no false positive or false negative observed, as previously described (Garciglia-Mercado *et al*. companion paper).

Conclusions

This study demonstrated the potential use of AA in critical hospital areas through the LAMP assays developed for detecting CRAB, which promises to be a cheap and efficacious disinfectant alternative to both developed and especially developing countries, preventing the spread of this organism in the environment and to other susceptible patients in hospital settings.

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