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Evaluation of a Loop-Mediated Isothermal Amplification-Based Methodology To Detect Carbapenemase Carriage in *Acinetobacter* Clinical Isolates

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Carbapenem-resistant *Acinetobacter baumannii* is a major source of nosocomial infections worldwide and is mainly associated with the acquisition of OXA-type carbapenemases and, to a lesser extent, metallo-β-lactamases (MBLs). In this study, 82 nonepidemiologically related *Acinetobacter* strains carrying different types of OXA or MBL enzymes were tested using the Eazyplex system, a loop-mediated isothermal amplification (LAMP)-based method to rapidly detect carbapenemase carriage. The presence/absence of carbapenem-hydrolyzing enzymes was correctly determined for all isolates in <30 min.

Acinetobacter baumannii is considered a nosocomial pathogen responsible for a wide spectrum of infections, affecting critically ill patients and causing high mortality rates in the intensive care unit (ICU) (1). Multidrug-resistant *A. baumannii* strains are increasingly common, thus challenging the choice for optimal treatment (2). The use of carbapenems in combined therapy is considered the treatment of choice for such infections, but resistance to this group of antibiotics has increased during the last decade and needs to be carefully monitored (1, 3). In Spain, only 14 to 18% of the *A. baumannii* isolates recovered in 2010 were carbapenem susceptible, showing a significant decrease compared to 2000, when up to 60% of all isolates were susceptible (4, 5).

The mechanisms involved in carbapenem resistance in *A. baumannii* include carbapenemase production, the reduced expression of outer membrane proteins, the overexpression of efflux pump(s), and altered penicillin-binding proteins (6). Among these mechanisms, the production of carbapenem-hydrolyzing class D β-lactamases is by far the most common carbapenem resistance mechanism worldwide, followed by the acquisition of class B metallo-β-lactamases and, less frequently, class A carbapenemases, such as *Klebsiella pneumoniae* carbapenemase (KPC) (7).

Carbapenem-hydrolyzing oxacillinases are divided into six groups: OXA-51/-69, which is chromosomally encoded in *A. baumannii*, and five clusters of acquired OXA enzymes (OXA-23, OXA-40, OXA-58, OXA-143, and OXA-235) (7–9). OXA-23 and OXA-58 are reported worldwide (1, 10), while OXA-40 is highly prevalent in Spain, Portugal, and Croatia (11–13). Enzymes belonging to the OXA-143 and OXA-235 clusters have recently been associated with North, Central, and South America (8, 9). Despite being less widespread, metallo-β-lactamases also contribute to carbapenem resistance in *Acinetobacter* spp., although only the IMP, VIM, SIM, and NDM types have been described in this genus so far (6). Of note, NDM-producing *Acinetobacter* spp. currently constitute an emerging threat in Asia and the Middle East (14).

The rapid detection of carbapenemase-producing *Acinetobacter* isolates is fundamental for adequate empirical treatment and infection control purposes. The Eazyplex system (Amplex-Diagnostics GmbH, Germany) combines loop-mediated isothermal amplification (LAMP) of the target and real-time photometric detection of amplified material for rapid and simple detection of carbapenemase-encoding genes (15). The aim of this study was to evaluate the efficacy of Eazyplex for detecting carbapenemase carriage in a well-defined collection of *Acinetobacter* clinical isolates.

Eighty-two nonepidemiologically related strains of *Acinetobacter* spp. were included to represent the most common carbapenemases likely encountered within this genus: 15 carbapenem-susceptible *A. baumannii* strains harboring only the chromosomally encoded OXA-51 enzyme, 19 *A. baumannii* strains containing the $bla_{\text{OXA-40}}$ gene, 24 *A. baumannii* strains containing $bla_{\text{OXA-58}}$, 20 *A. baumannii* strains with $bla_{\text{OXA-23}}$, 3 additional strains carrying NDM enzymes (1 NDM-2-producing *A. baumannii* and 2 NDM-1-producing *A. pittii*), and 1 *A. baumannii* strain harboring $bla_{\text{IMP-2}}$. The species identifications of all isolates were confirmed by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (16) and amplified rRNA gene restriction analysis (ARDRA) (17). The MICs of imipenem and meropenem were determined using Etest strips (bioMérieux, Madrid, Spain) and interpreted according to EUCAST guidelines (version 4.0, 2014 [http://www.eucast.org]). The presence of genes encoding carbapenemases was confirmed by PCR and DNA sequencing (18–21), with all strains but the two from *A. pittii* being positive for $bla_{\text{OXA-51}}$, which is in good agreement with previous reports indicating intrinsic carriage of $bla_{\text{OXA-51}}$ in *A. baumannii* (22).

All strains were tested using the Eazyplex SuperBug complete strips, covering KPC, NDM, OXA-48, VIM, OXA-23, OXA-40, and OXA-58, run on a Genie II instrument (OptiGene, Horsham,

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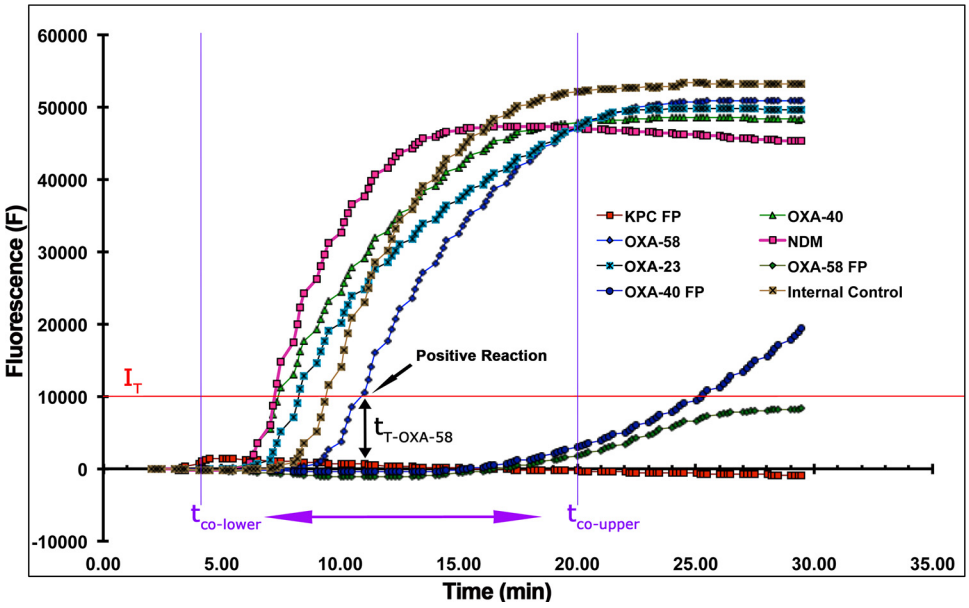

FIG 1 Loop-mediated isothermal amplification (fluorescence arbitrary units [F]) over time of carbapenemase-encoding genes from representative *Acinetobacter* spp. strains harboring OXA-23, OXA-40, OXA-58, and NDM enzymes. I_T, fluorescence intensity threshold; t_T, threshold time; $t_{co\text{-lower}}$ and $t_{co\text{-upper}}$, lower and upper cutoff time values, respectively; FP, false positive. The black arrow indicates the t_T value for the OXA-58 reaction that defines a positive result.

United Kingdom) for target isothermal amplification and detection. A single bacterial colony for each strain was suspended in 500 μl of resuspension and lysis fluid (RALF) and incubated in a thermal block (99°C) for 2 min. Fifty milliliters of the cell suspension was incubated in blood agar before lysis to test for bacterial contamination. Twenty-five milliliters of the RALF suspension was added to each tube of the strip containing the ready-to-use mastermix. Air bubbles were removed by gently knocking the tubes, and each strip was immediately placed into the Genie II instrument. The reaction mixtures were incubated at 66°C for 30 min with fluorescence monitoring. Isothermal amplification was indicated by a strong increase in the fluorescence signal in the form of a typical amplification curve (Fig. 1). The Eazyplex SuperBug complete test strip contains eight caps with six oligonucleotide primers in each cap, allowing the simultaneous specific amplification of seven different resistance genes in a single run, plus an internal control. In the presence of the relevant DNA, the specific amplification products of the genes encoding KPC, NDM, VIM, OXA-48, OXA-23, OXA-40, and OXA-58 were synthesized and visualized using a real-time-fluorescence measurement of a fluorescence dye bound to double-stranded DNA. The presence or absence of carbapenemase-encoding genes was correctly identified for all isolates, except for the single *A. baumannii* strain harboring $bla_{\text{IMP-2}}$ and the presence of $bla_{\text{OXA-51}}$ in all strains, since the test strip does not include specific oligonucleotides to identify these two genes. Unspecific DNA amplification was observed for five samples in the KPC, OXA-58, and OXA-40 reaction tubes, which accounted for false-positive results. A set of parameters was defined according to the manufacturer's specifications in order to correctly assign positive/negative results to each individual LAMP reaction. Specifically, a fluorescence intensity threshold (I_T) value significantly above the background noise level was set at 10,000 fluorescence units to indicate when a reaction had produced double-stranded DNA (dsDNA) products; a threshold time (t_T) was defined as the time at which the real-time fluorescence value crossed the threshold intensity value; a lower cutoff time ($t_{co\text{-lower}}$) was set at 4 min, and an upper cutoff time ($t_{co\text{-upper}}$) was set at 20 min to define lower and upper limits of the acceptable threshold values. Isothermal amplification of target DNA was only considered truly positive when the fluorescence values for a specific reaction crossed the fluorescence intensity threshold within the range set by the cutoff times ($t_{co\text{-lower}} < t_T < t_{co\text{-upper}}$). Using these parameters, not only were false-positive amplifications easily identified, but they allowed the detection of true-positive results before the end of the run. The positive t_T values for all target genes and the internal control ranged between 7 and 15 min (Fig. 1). Overall, better results were achieved when a small fraction of a colony was used instead of fully loading a sterile loop, which either inhibited the amplification reaction or increased unspecific amplification (data not shown).

The Eazyplex test system allows the amplification and detection of target genes in a single step at a constant temperature and provides highly reliable results in <15 min. The implementation of this system in routine clinical laboratories provides clinicians with early valuable information for the accurate management of patients with infections caused by carbapenem-resistant *Acinetobacter* species. In addition, the Eazyplex SuperBug complete test has been validated by the manufacturer for the direct testing of rectal smears, suggesting that this system has the potential to test noncultured samples, such as those derived from body fluids. Detection, however, is limited to the current range of available oligonucleotides, and negative samples should be evaluated with alternative methods (antimicrobial susceptibility tests [AST] and conventional PCR) to detect the carriage of resistance genes not included in the assay. To allow reliable implementation in routine analysis, the Eazyplex system should be further expanded to include additional carbapenemase-encoding genes reported in *Acinetobacter* species.

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