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Imipenem–avibactam: a novel combination for the rapid detection of carbapenemase activity in *Enterobacteriaceae* and *Acinetobacter baumannii* by matrix-assisted laser desorption ionization-time of flight mass spectrometry

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ABSTRACT

In the present study, we propose a novel matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)-based method for detecting carbapenemase-producing *Enterobacteriaceae* and *Acinetobacter baumannii*. For this, we analyzed a series of 131 isolates. Among them, a total of 115 *Enterobacteriaceae*: 79 of them carrying a carbapenemase enzyme (15bla_{KPC}, 7bla_{NDM}, 11bla_{IMP}, 12bla_{VIM}, and 34bla_{OXA-48}) and 16 *A. baumannii* isolates: 15 of them carrying carbapenemases (10bla_{OXA-23}, 2bla_{OXA-58}, 2bla_{OXA-24}, and 1$bla_{OXA-237}$). The rest of the isolates were noncarbapenemase producers and used as negative controls. The isolates were submitted to susceptibility testing using a combination of imipenem–avibactam and analysis by the MALDI-TOF Biotyper Compass software (Bruker Daltonik, Germany). The assay showed an overall sensitivity and specificity for carbapenemase detection of 98% and 100%, respectively. The combination of imipenem and avibactam displayed activity against KPC and OXA-48-producing *Enterobacteriaceae* and thus represents a new strategy for identifying and confirming these carbapenemases. However, the combination did not provide any benefit over *A. baumannii*.

1. Introduction

The spread of carbapenemase enzymes in *Enterobacteriaceae* and *A. baumannii* has led to an emerging health concern because of the difficulty in detection and control (Nordmann and Poirel, 2014; World Health Organization, 2014) and especially because of the lack of adequate antimicrobial therapy (Petrosillo et al., 2013). Although β-lactamase inhibitors have played an important role in combating β-lactam resistance in gram-negative bacteria, their effectiveness has diminished as diverse varieties of β-lactamases have emerged. Novel β-lactamase inhibitors are being developed with the aim of restoring the activity of β-lactam antibiotics against carbapenemase-producing *Enterobacteriaceae* (Drawz et al., 2014; Toussaint and Gallagher, 2015). Avibactam displays potent, broad-spectrum inhibition of Ambler class A and class C β-lactamases and some inhibitory capacity against class D enzymes (Zhanel et al., 2013). The ceftazidime–avibactam (CAZ-AVI) combination has recently undergone testing and further studies are currently under way (Mazuski et al., 2016). Numerous publications have confirmed the in vitro effectiveness of the CAZ-AVI combination against CTX-M, KPC, and OXA-48-type producers (Aktaş et al., 2012; Levasseur et al., 2015; Sader et al., 2014) and the poor activity toward OXA-10 and OXA-24-producers (Ehmann et al., 2013; Lahiri et al., 2015). Other combinations with avibactam are being developed (e.g., ceftaroline–avibactam and aztreonam–avibactam), and show promising results (Bush, 2015; Li et al., 2015).

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has become an established tool for the rapid identification of microorganisms in microbiological laboratories (Barba et al., 2014; Carbonnelle et al., 2011; El-Bouri et al., 2012). The technique has recently proved useful for detecting β-lactamase activity, based on monitoring the mass peaks of the hydrolyzed and nonhydrolyzed forms of the antibiotics, which differ in their molecular weight (Jung et al., 2014; Lasserre et al., 2015; Oviaño et al., 2014, 2016a; Papagiannitsis et al., 2015; Sparbier et al., 2012).

With the aim of continuing the evaluation of new effective combinations of β-lactams and avibactam, we developed a novel approach for the detection of carbapenemase-producing isolates using an imipenem–avibactam (IMI-AVI) assay with a series of 131 bacterial isolates, 94 of which are carbapenemase producers. Carbapenemase resistance can be detected after 30-minute incubation with imipenem and further evaluation with an automated software algorithm (MALDI-TOF Biotyper Compass software; Bruker Daltonik). Imipenem was chosen as the antibiotic marker for carbapenem resistance as it displays broad spectrum activity against both *Enterobacteriaceae* and *A. baumannii*, and it also shows to our concern the highest turnover rate of β-lactam hydrolysis. This approach makes use of the capacity of avibactam to inhibit imipenem hydrolysis toward different types of carbapenemase

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producers in an assay with *Enterobacteriaceae* and *A. baumannii* and rapid detection by MALDI-TOF MS.

2. Material and methods

2.1. Bacterial isolates

A total of 131 previously characterized nonrepeat *Enterobacteriaceae* and *A. baumannii* isolates from Spain were tested (Supplementary Material). Of the 115 *Enterobacteriaceae* isolates, 79 expressed a carbapenemase enzyme (15bla_{KPC}, 7bla_{NDM}, 11bla_{IMP}, 12bla_{VIM}, and 34bla_{OXA-48}) and the other 36 isolates, which expressed resistance mechanisms other than carbapenemases, were used as negative controls. These controls comprised 9 β-lactamase-producing isolates (4 bla_{SHV} and 5 bla_{K1}), 12 AmpC-type (3 bla_{EBC}, 1 bla_{FOX}, 3 bla_{CMY}, and 5 bla_{DHA}), 14 ESBL-type (14 $bla_{CTX-M-type}$) isolates, and 1 isolate that remained fully susceptible to cephalosporins and did not express AmpC-type, ESBL-type, or any other β-lactamase or carbapenemase enzyme. Of the 16 *A. baumannii* isolates included in the study, 15 of them expressed carbapenemases (10bla_{OXA-23}, 2bla_{OXA-58}, 2bla_{OXA-24}, and 1$bla_{OXA-237}$) and 1 isolate was fully susceptible to all antibiotics. The *Enterobacteriaceae* isolates comprise 66 *Klebsiella pneumoniae*, 11 *K. oxytoca*, 17 *Escherichia coli*, 18 *Enterobacter cloacae*, 1 *E. aerogenes*, and 2 *Citrobacter freundii*. All strains were characterized in relation to carbapenemases, ESBLs, and AmpC-type enzymes by polymerase chain reaction and sequencing.

2.2. MBT STAR-BL assay

The MALDI Biotyper Selective Testing of Antibiotic Resistance β-lactamase (MBT STAR-BL) assay was performed as described by Sparbier et al. (2012). Briefly, for analysis of plated bacteria, a 1-μl loop of bacteria was resuspended in 50 μl of reaction buffer. Bacteria were evaluated simultaneously in a solution containing imipenem (0.25 mg/mL, Sigma-Aldrich, Germany; 10 mM NH_4HCO_3, 0.005% SDS, pH 8.0) (Oviaño et al., 2016b) and in a solution containing the combined IMI-AVI solution (25 μl of imipenem 0.50 mg/mL and 25 μl of avibactam; 10 mM NH_4HCO_3, 0.005% SDS, pH 8.0). The final concentration of imipenem in the combined antibiotic solution was maintained so that the hydrolysis rate of the isolates in the combined solution could be compared with the imipenem solution alone. The influence of the concentration of avibactam (AstraZeneca AB, Sweden: 1.5, 3, and 4 mg/mL) was also evaluated. The bacteria and antibiotic solutions were incubated at 37 °C for 30 minutes under agitation.

2.3. MALDI-TOF MS analysis

For MALDI-TOF MS measurements, duplicate samples (1 μl) of the supernatant were spotted directly onto a polished steel MALDI target plate. Dried spots were overlaid with MALDI matrix (10 mg/mL of α-cyano-4-hydroxy-cinnamic acid [α-HCCA] in 50% acetonitrile, 0.1% trifluoroacetic acid; Bruker Daltonik) containing a modified reserpine represented by the mass of 607.26 Da as an internal standard. Once the matrix was dry, mass spectra were acquired in a microflex LT/SH bench-top mass spectrometer (Bruker Daltonik) equipped with a 60 Hz nitrogen laser. Measurements were made in the mass range between 100 and 1000 Da by employing an optimized acquisition imipenem method in the STAR-BL module in FlexControl 3.3 (Bruker Daltonik GmbH).

2.4. Data evaluation

MALDI-TOF Biotyper Compass software (Bruker Daltonik) was used to evaluate the spectra. The software automatically calculates the logRQ value (which indicates the rate of hydrolysis) for imipenem as the logarithmic ratio of the intensity of the internal standard peak and the intensity of the non-hydrolyzed imipenem. LogRQ values were normalized according to defined negative and positive control strains. Normalized LogRQ values similar to or below 0.2 represent negative strains. Normalized LogRQ values similar to or above 0.4 indicate β-lactamase activity. Normalized LogRQ values between 0.2 and 0.4 represent an ambiguous hydrolysis pattern which requires further testing.

3. Results

3.1. Imipenem performance for carbapenemase detection

Imipenem performance has previously been optimized in a paper by our group (Oviaño et al., 2016b). Following these conditions, we performed the assay. After incubation of isolates for 30 minutes, we observed imipenem hydrolysis in all except 2 *A. baumannii* bla_{OXA-58} strains. These results can be explained by the lower carbapenems MICs than these isolates use to display comparing to other *A. baumannii* OXA enzymes. The sensitivity and specificity imipenem hydrolysis assay were 98% (92/94) and 100% (37/37), respectively.

3.2. Optimization of the concentration of avibactam

The reaction conditions were optimized in relation to the concentration of avibactam required for inhibition of imipenem hydrolysis and a proper MALDI-TOF MS spectra acquisition. For this purpose, we performed the β-lactamase assay with a set of 8 isolates chosen at random from those expressing a KPC or OXA-48-type carbapenemase enzyme, in the presence of 3 different concentrations of avibactam (1.5, 3, and 4 mg/mL) (Fig. 1). Use of 1.5 mg/mL of avibactam resulted in poor inhibition of imipenem hydrolysis. The logRQ value for imipenem hydrolysis decreased but was still higher than 0.4 for almost all (7/8) isolates. Use of 3 mg/mL avibactam yielded a logRQ value of between 0.1 and 0.4 for all isolates, indicating ambiguous inhibition that results in a substantial decrease in imipenem hydrolysis but that is not sufficient to ensure complete inhibition. Finally, use of 4 mg/mL avibactam led to complete inhibition of imipenem hydrolysis in all the isolates tested (8/8), ensuring activity of the antibiotic.

3.3. Avibactam inhibition assay

The inhibition assay was performed with 4 mg/mL of avibactam. Under these previously optimized conditions, we observed significant modification of the imipenem hydrolysis ratio when the combined IMI-AVI solution was applied to KPC-type enzymes (Fig. 2; Supplementary Material). In all isolates tested (15/15), avibactam was able to inhibit the hydrolytic effect of the enzyme, thus restoring the imipenem activity. In all cases, the IMI-AVI logRQ value was below 0.2 and the average value was −0.2 when avibactam was applied to the reaction solution. The opposite effect was observed in the isolates expressing metallo-β-lactamases (Fig. 2; Supplementary Material). In all cases, the IMI-AVI logRQ value was higher than 0.8 and the average value was 1.3 (Supplementary Material), so in this case the avibactam was unable to inhibit the action of the metallo-β-lactamases on imipenem (0/30). For group D carbapenemase enzymes, the behavior differed depending on the type of β-lactamase. Avibactam was able to inhibit the action of OXA-48-type enzymes, and the IMI-AVI logRQ value was below 0.2 in all cases (34/34) and the average value was −0.3 (Fig. 2; Supplementary Material). For other OXA-type enzymes (other than bla_{OXA-48}) belonging to *A. baumannii*, the opposite result was obtained, and avibactam was unable to inhibit the hydrolytic effect of these enzymes (Fig. 2; Supplementary Material). The IMI-AVI logRQ value was above 0.4 in all cases (13/13) and the average value was 0.8. The average decrease in imipenem hydrolysis in the presence of avibactam was 0.1, indicating that the logRQ values for the isolates in presence and absence of avibactam were almost the same, and imipenem hydrolysis was not reduced. None of the negative controls displayed a positive hydrolysis