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Original article

Detection of *bla*_{OXA-23} in *Acinetobacter* spp. isolated from patients of a university hospital

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ABSTRACT

Introduction: *Acinetobacter* spp. have emerged as notorious pathogens involved in healthcare-associated infections. Carbapenems are important antimicrobial agents for treating infections due to multidrug resistant *Acinetobacter* spp. Different mechanisms may confer resistance to these drugs in the genus, particularly production of class D carbapenemases. OXA-23-like family has been pointed out as one of the predominant carbapenemases among *Acinetobacter*. The present work aimed to investigate the occurrence of OXA-23-like carbapenemases among *Acinetobacter* isolates recovered from patients of a university hospital in Niterói, RJ, Brazil.

Methods: Antimicrobial susceptibility profiles were determined by disk-diffusion. Imipenem resistant isolates were submitted to Modified Hodge Test in order to screen for carbapenemase production, and later to polymerase chain reaction (PCR) to investigate the presence of *bla*_{OXA-23}.

Results: Imipenem and meropenem resistance rates were 71.4% and 69.7%, respectively. The Modified Hodge Test revealed carbapenemase production among 76 (89.4%) of the 85 imipenem resistant isolates analyzed; according to PCR results, 81 isolates (95.4%) carried the *bla*_{OXA-23} gene.

Conclusions: OXA-23-like enzymes may be an important mechanism of carbapenem resistance among isolates present in the hospital studied.

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Introduction

Acinetobacter spp. have emerged as one of the most important pathogens involved in health care associated infections in recent decades. These non-fermentative Gram-negative cocobacilli are frequently involved in the etiology of ventilator-associated pneumonia, bacteremia, urinary tract infections, and surgical site infections. These pathogens are also notorious for their ability to accumulate different mechanisms of antimicrobial resistance, often showing a multidrug-resistant phenotype.¹⁻⁴

Carbapenems are considered important antimicrobial agents for treating infections due to multidrug-resistant *Acinetobacter* spp. However, reports of resistance to these drugs have emerged, with increasing frequency, among *Acinetobacter* spp. clinical isolates.⁵⁻¹⁰

Different mechanisms may confer carbapenem resistance in *Acinetobacter* spp., but production of carbapenemases is considered the most important one, particularly those belonging to Ambler's class D, also known as oxacilinases (OXA). The class B carbapenemases or metallo- β -lactamases (M β LS) can also be found among *Acinetobacter* spp., although less frequently.¹¹

Five major groups of OXA with carbapenemase activity have been identified in *Acinetobacter baumannii*: acquired OXA-23-like, OXA-40-like, OXA-58-like, and OXA-143-like families, and the OXA-51 group, which codifies a chromosomal oxacilinase intrinsic to *A. baumannii*. When overexpressed, these enzymes can confer carbapenem resistance.¹²⁻¹⁷

Among OXA, the variants comprising the OXA-23-like family have been detected throughout the world, and have also been pointed out as the predominant carbapenemases among *Acinetobacter* in several geographic regions.

In Brazil, the first report of isolates producing these enzymes were in the city of Curitiba, Paraná.^{15,18-23} After that report, *Acinetobacter* spp. clinical isolates producing OXA-23-like enzymes have been also identified in cities like Porto Alegre-RS, Rio de Janeiro-RJ, Niterói-RJ, São Paulo-SP, Belo Horizonte-MG, Blumenau-SC, and São Luís-MA.²⁴⁻³¹ The OXA-143-like enzymes, as well as OXA-72 and OXA-58, have also been found in Brazil.^{12,16,17,27,32}

The present study aimed to investigate the occurrence of isolates producing the OXA-23-like carbapenemases among imipenem and/or meropenem-resistant *Acinetobacter* isolates, isolated at a university hospital in Niterói, RJ, Brazil.

Material and methods

Bacterial strains

A total of 119 *Acinetobacter* spp. clinical isolates were studied. They were recovered between July 2007 and July 2009, from different clinical specimens of 100 patients admitted to Hospital Universitário Antônio Pedro (HUAP), a 290-bed public tertiary teaching hospital. Isolates recovered from the same patient that were isolated from different infection sites, at different periods of time, or that showed distinct antimicrobial susceptibility profiles were included in the study.

The most frequent source of isolation was the inferior respiratory tract (n = 44; 37%), followed by blood (n = 28; 23.5%), urine (n = 17; 14.3%), and catheter tip (n = 14; 11.8%). Skin and ascitic fluid isolates corresponded to 5% (n = 6) and 2.5% (n = 3) of the entire collection, respectively. Other specimens represented 4.2% (n = 5), whereas two isolates could not have their origin determined (1.7%). The intensive care unit (ICU) was the hospital ward that showed the greatest number of isolates (68/119; 57.1%).

Bacterial strains identification

The identification of the isolates at the genus level was performed using the VITEK 1 (bioMérieux - Marcy l'Etoile, France) automated system, at the Microbiology Laboratory of the Pathology Service of HUAP.

Antimicrobial susceptibility profiles

The antimicrobial susceptibility profiles of clinical isolates were determined by disk-diffusion, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI - 2009).³³ The following antimicrobials (CECON - São Paulo, Brazil) were tested: amikacin (30 μ g), ampicillin/sulbactam (10 μ g), cefepime (30 μ g), ceftazidime (30 μ g), ciprofloxacin (5 μ g), gentamicin (10 μ g), imipenem (10 μ g), meropenem (10 μ g), piperacillin/tazobactam, (75 mg/10 μ g), sulfamethoxazole/trimethoprim (23.75/1.25 μ g), ticarcillin/clavulanate (75/10 μ g), and tetracycline (30 μ g). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains.

Phenotypic detection of carbapenemases production

Imipenem resistant (IMP-R) isolates were submitted to Modified Hodge Test, as described previously by Lee et al. (2001), with the modifications indicated below.³⁴

The surface of a Mueller-Hinton agar plate was inoculated with a bacterial suspension made of *E. coli* ATCC 25992 fresh cultures (0.5 McFarland turbidity). A 10 μ g imipenem disk was placed at the center of the plate. Then, streaks of the imipenem-resistant isolates were performed from the edge of the disk to the edge of the plate. The test result was considered positive if the presence of a distorted inhibition zone was observed after incubation at 37°C during 16 h to 18 h. *Acinetobacter* spp. strain A29009 producing OXA-23 was used as positive control.

Detection of *bla*_{OXA-23}

All imipenem resistant isolates were investigated by PCR in order to determine the presence of *bla*_{OXA-23}, according to Woodford et al. with the modifications described below.³⁵

DNA extraction was accomplished by boiling a 3.0 McFarland turbidity suspension of each isolate for 10 minutes. Then, these suspensions were centrifuged at 8,000 rpm for 2 minutes and the supernatant was used as template in PCR experiments. A 2 μ L volume of DNA template was added to 23 μ L of reaction mixture, which contained 2.5 μ L of PCR buffer 10x (10 mM Tris HCl, 25 mM KCl), 1 mM of MgCl₂, 0.5 μ M of

Table 1 – Antimicrobial susceptibility profile of 119 *Acinetobacter* spp. clinical isolates recovered between July 2007 and July 2009, at the Hospital Universitário Antônio Pedro, in Niterói, RJ, Brazil.

Antimicrobial	Resistant n (%)	Intermediate n (%)	Sensitive n (%)
Amikacin	41 (34.5)	8 (6.7)	70 (58.8)
Ampicillin/sulbactam	50 (42)	21 (17.7)	48 (40.3)
Cefepime	98 (82.3)	2 (1.7)	19 (16)
Ceftazidime	92 (77.3)	2 (1.7)	25 (21)
Ciprofloxacin	103 (86.6)	-	16 (13.4)
Gentamicin	68 (57.1)	-	51 (42.9)
Imipenem	85 (71.4)	-	34 (28.6)
Meropenem	83 (69.7)	-	36 (30.3)
Piperacillin/tazobactam	91 (76.5)	2 (1.7)	26 (21.8)
Sulfamethoxazole/trimethoprim	94 (79)	4 (3.4)	21 (17.6)
Ticarcillin/clavulanate	95 (79.8)	2 (1.7)	22 (18.5)
Tetracycline	14 (11.8)	39 (32.8)	66 (55.4)

each primer, 100 μ M of each deoxynucleotide trifosfate (dATP, dGTP, dCTP, dTTP) and 1.5U of Taq polimerase (Invitrogen – São Paulo, Brazil). The sequences of primers used were: GATCG-GATTGGAGAACCAGA and ATTTCTGACCGCATTCCAT.³⁰The reaction was carried as follows: initial denaturation at 94 °C for 5 minutes; followed by 30 cycles of 94 °C for 1 minute, 54 °C for 1 minute, 72 °C for 1 minute; and final extension of 72 °C for 5 minutes. A PCR product of 501pb was expected. *Acinetobacter* spp. strain A 29009 producing OXA-23 was used as positive control for the amplification experiments.

Results

Antimicrobial susceptibility profiles

High rates of resistance were observed among isolates studied. Resistance rates $\geq 70\%$ were observed for seven of the 12 antimicrobial agents tested (Table 1). The highest resistance

rate was observed for ciprofloxacin (86.6%), followed by cefepime (82.3%). The percentages for carbapenems were also elevated; 71.4% for imipenem and 69.7% for meropenem.

The isolates showed the highest susceptibility rates to amikacin, followed by tetracycline and gentamicin (58.8%, 55.4%, and 42.9%, respectively). Of notice, a relatively high rate of intermediate resistance was observed for tetracycline (32.8%) and ampicillin/sulbactam (17.7%).

IMP-R strains were isolated throughout the entire study period and showed more elevated resistance rates to other antimicrobials than isolates susceptible to this antimicrobial (Fig. 1). Among IMP-R isolates, the highest susceptibility rate was also observed for amikacin (60%).

Most carbapenem resistant isolates were recovered from patients hospitalized at the ICU (80%; 68/85), and were recovered mostly from the inferior respiratory tract, (n = 35; 41.2%), followed by blood (n = 21; 25.7%), urine (n = 11; 12.9%), and catheter tip (n = 9; 10.6%).

Detection of carbapenemase production and bla_{OXA-23}

The Modified Hodge Test results revealed carbapenemase production among 76 (89.4%) of the 85 IMP-R *Acinetobacter* isolates analyzed (Fig. 2).

According to results obtained by PCR, 81 (95.4%) of the 85 IMP-R isolates carried the bla_{OXA-23} gene (Fig. 3).

Six isolates carrying bla_{OXA-23} showed negative results for modified Hodge Test. Conversely, one isolate was positive for this phenotypic test, but the presence of bla_{OXA-23} was not detected. One of the two isolates that were susceptible to meropenem and resistant to imipenem carried the bla_{OXA-23} gene.

Discussion

All isolates evaluated in this study have been identified as belonging to the *A. baumannii*-complex by the automated system. However, it is well established that this method is not

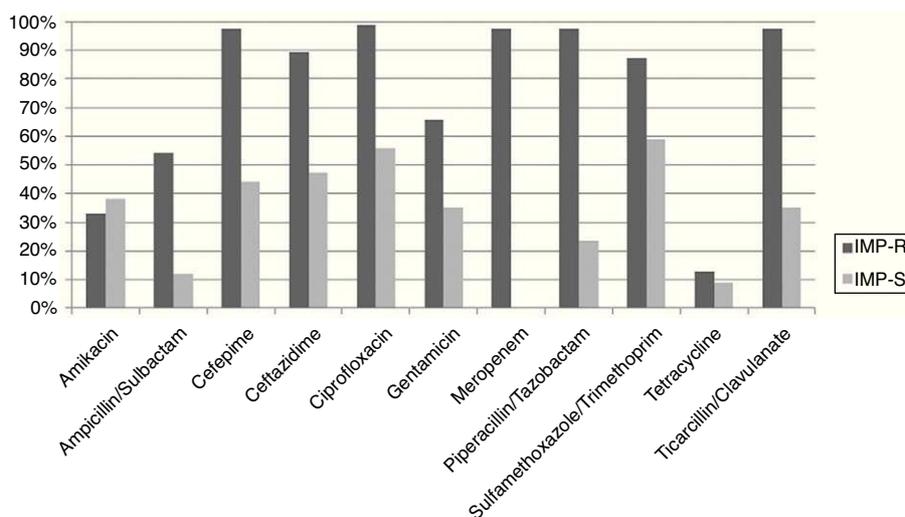


Fig. 1 – Comparison between the antimicrobial resistance profile of 85 imipenem-resistant and 34 imipenem-susceptible *Acinetobacter* spp. clinical isolates recovered between July 2007 and July 2009, at the Hospital Universitário Antônio Pedro, in Niterói, RJ, Brazil.

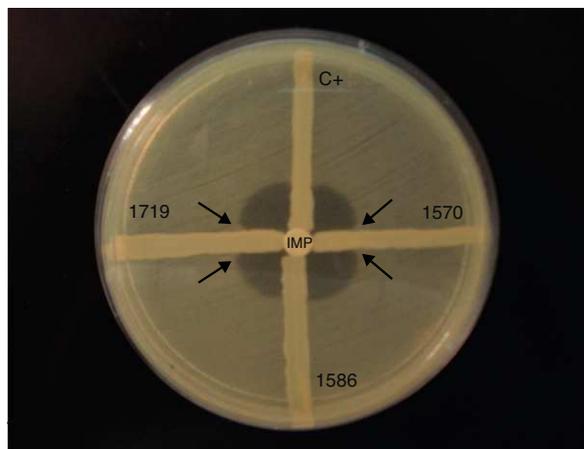


Fig. 2 – Modified Hodge Test results for *Acinetobacter* spp. clinical isolates. Positive results are indicated with an arrow. C+, positive control; IMP, imipenem disk; 1570, 1586, and 1719 are imipenem-resistant *Acinetobacter* spp.

suitable to distinguish between *A. baumannii* and other species such as *Acinetobacter pitii*, *Acinetobacter nosocomialis*, and *Acinetobacter calcoaceticus*.³⁶ The presence of *bla*_{OXA-51} in all isolates was also investigated, with positive results (data not shown). Although *bla*_{OXA-51} detection has been pointed out as a simple and reliable way of identifying *A. baumannii*, this gene has also been found in *A. nosocomialis*, jeopardizing this presumptive bacterial identification at the species level.^{37,38} For the abovementioned reasons, bacterial identification results are expressed only at the genus level in this study.

High resistance rates were observed for most of the antimicrobials agents studied, including carbapenems, and even more elevated rates were observed among IMP-R isolates. Antimicrobial resistance considerably restricts the available treatment options, especially resistance to carbapenem, which is considered to be the first option to treat severe infections due to *Acinetobacter* spp.

Modified Hodge Test results showed good correlation with PCR (96.2%), since only six *bla*_{OXA-23}-positive isolates could not be detected using the phenotypic methodology, suggesting

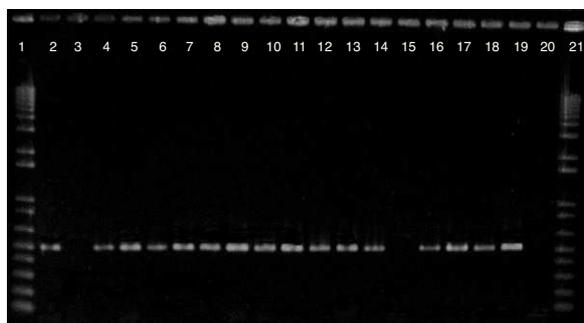


Fig. 3 – Detection of *bla*_{OXA-23}-like genes in *Acinetobacter* spp. clinical isolates. Lines 1 and 21: 1 Kb Plus DNA Ladder; line 2: *bla*_{OXA-23} positive control; line 3: *bla*_{OXA-23} negative control; lines 4-14 and 16-19: isolates carrying *bla*_{OXA-23}; line 15: isolate not carrying *bla*_{OXA-23}; line 20: reaction control.

that this phenotypic technique is appropriate for screening *Acinetobacter* spp. isolates producing such carbapenemases. These results were consistent with those of Yang et al, which observed 100% positivity in Modified Hodge Test among *A. baumannii* strains that were IMP-R and OXA-23 producers.²⁰ Recently, researchers from Rio de Janeiro pointed out that 87.6% of IMP-R isolates carrying *bla*_{OXA-23} were detected as carbapenemase producers by the Modified Hodge Test.²⁴

In the present study, one isolate showing positive Modified Hodge Test result did not carry the *bla*_{OXA-23} gene, which could suggest either a false-positive phenotypic result or the presence of another carbapenemase type. Metallo-β-lactamases may be involved in carbapenem resistance phenotype among *Acinetobacter* spp., although less frequently than class D enzymes. In addition, coproduction of metallo-beta-lactamases and oxacilinases has been described in *Acinetobacter* spp. In this study, phenotypic screening tests were conducted to assess the production of metallo-β-lactamases,³⁹ although no isolate showed positive results (data not shown). Thus, the mechanism involved in carbapenem resistance in the abovementioned isolate remains to be elucidated.^{40,41}

A high frequency of *bla*_{OXA-23} (95.4%) was observed among IMP-R isolates analyzed. This result implies that production of OXA-23-like enzymes may be an important mechanism of carbapenem resistance among isolates present in the hospital studied.

The detection of *A. baumannii* producing OXA-23 is frequent in many countries, suggesting a worldwide dissemination of this enzyme.²¹ According to Kempf and Rolain, Europe, Singapore, Australia, the USA, Algeria, Egypt, Libya, South Africa, Thailand, Tunisia, Iraq, and French Polynesia reported either nosocomial outbreaks or sporadic cases of *Acinetobacter* producing OXA-23.⁴² In Asia, the most prevalent disseminated class D enzyme amongst *A. baumannii* in China and Korea is OXA-23.⁴³ In Latin America, OXA-23 was identified in *A. baumannii* isolates from Argentina, Colombia, and Brazil.³⁰ In Brazil, OXA-23-like enzymes have been identified as one of the major carbapenem resistance mechanism in *Acinetobacter* spp.^{25,28-32,44,45} In Rio de Janeiro, a surveillance study conducted in eight hospitals showed a wide dissemination of isolates producing such carbapenemases. In Niterói, the occurrence of *A. baumannii* strains carrying *bla*_{OXA-23} has already been reported at a public hospital, however, at a smaller rate (15%) than found in the present study.^{24,27}

In conclusion, early detection of IMP-R *Acinetobacter* isolates, and of the corresponding mechanism of resistance is extremely important to prevent their dissemination throughout the hospital environment. It could be beneficial to routinely perform the surveillance of isolates producing carbapenemases in the hospital investigated. In addition, strict control measures, as well as prudent utilization of antimicrobials agents should be continuously promoted.

Conflict of interest

All authors declare to have no conflict of interest.

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