Detection of blaOXA-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 carbapenamases 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 blaOXA-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 blaOXA-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.  

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.  

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 (MβLs) can also be found among Acinetobacter spp., although less frequently.  

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 oxacilnase intrinsic to A. baumannii. When overexpressed, these enzymes can confer carbapenem resistance.  

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.  

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). The following antimicrobials (CECON – São Paulo, Brazil) were tested: amikacin (30 μg), ampicillin/sulbactam (10 μg), ceftazidime (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), imipenem (10 μg), meropenem (10 μg), piperacillin/tazobactam, (75 mg/10 μg), sulfamethoxazole/trimethoprim (23.75/1.25 μg), ticarcillin/clavulanate (75/10 μg), and tetracycline (30 μg). 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 bellow.  

Detection of blaOXA-23

All imipenem resistant isolates were investigated by PCR in order to determine the presence of blaOXA-23, according to Woodford et al. with the modifications described below.  

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 μL volume of DNA template was added to 23 μL of reaction mixture, which contained 2.5 μL of PCR buffer 10x (10 mM Tris HCl, 25 mM KCl), 1 mM of MgCl2, 0.5 μM 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.

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

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.

**Results**

Antimicrobial susceptibility profiles

High rates of resistance were observed among isolates studied. Resistance rates ≥ 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 blaOXA-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 blaOXA-23 gene (Fig. 3).

Six isolates carrying blaOXA-23 showed negative results for modified Hodge Test. Conversely, one isolate was positive for this phenotypic test, but the presence of blaOXA-23 was not detected. One of the two isolates that were susceptible to meropenem and resistant to imipenem carried the blaOXA-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
In Latin America, OXA-23 was identified in Brazil, although no isolate showed positive results. In Asia, the most prevalent dissemination due to carbapenemase producers by the Modified Hodge Test. Recently, researchers from Rio de Janeiro pointed out that 87.6% of IMP-R isolates carrying blaOXA-23 were detected as carbapenemase producers by the Modified Hodge Test. In the present study, one isolate showing positive Modified Hodge Test result did not carry the blaOXA-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, although no isolate showed positive results (data not shown). Thus, the mechanism involved in carbapenem resistance in the abovementioned isolate remains to be elucidated. A high frequency of blaOXA-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. 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. In Asia, the most prevalent disseminated class D enzyme amongst A. baumannii in China and Korea is OXA-23. In Latin America, OXA-23 was identified in A. baumannii isolates from Argentina, Colombia, and Brazil. In Brazil, OXA-23-like enzymes have been identified as one of the major carbapenem resistance mechanisms in Acinetobacter spp. 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 blaOXA-23 has already been reported at a public hospital, however, at a smaller rate (15%) than found in the present study.

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.
Acknowledgements

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