Old Clinical Isolates of Acinetobacter seifertii in Brazil Producing OXA-58

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The genus Acinetobacter comprises several groups of closely related species, including the Acinetobacter calcoaceticus-Acinetobacter baumannii complex (1) and a number of hemolytic species (2). Currently, the A. calcoaceticus-A. baumannii complex comprises five validly named species, including A. calcoaceticus, A. baumannii, A. pittii, and A. nosocomialis (3); the recently named species A. seifertii (4); and the yet-unnamed genomic species ‘between 1 and 3’ (5). A. seifertii has been shown to be responsible for causing human infections, thus representing an emergent pathogen (6–8). In the present study, we characterized two ancient carbapenem-resistant A. seifertii clinical isolates carrying the blaOXA-58 gene in Brazil.

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During a local surveillance study, two carbapenem-resistant Acinetobacter isolates (Asp-1069 and Asp-70041) had been recovered from patients hospitalized in a tertiary teaching hospital in the city of São Paulo, Brazil, in the early 1990s (Table 1). The isolates, originally assigned to the A. calcoaceticus-A. baumannii complex by phenotypic methods, were identified as A. seifertii by rpoB sequencing (9). Antimicrobial susceptibility testing was performed by CLSI broth microdilution (10), except for the tigecycline test, which was performed by Etest (bioMérieux, Marcy l’Etoile, France), according to the manufacturer’s recommendations (Table 1). Both strains were highly resistant to ampicillin–sulbactam (MICs, 64/32 mg/liter), third- and fourth-generation cephalosporins (MICs ranging from >32 to >128 mg/liter), imipenem (MICs ranging from 32 to >32 mg/liter), meropenem (MICs ranging from 32 to >32 mg/liter), and amikacin (MICs, >128 mg/liter) (11). The Asp-1069 strain was resistant to gentamicin (MIC, >64 mg/liter) and polymyxin B (MIC, 4 mg/liter), while Asp-70041 was susceptible to both of these drugs (MICs, 4 and 1 mg/liter, respectively). The most active antimicrobial agent tested against Asp-1069 and Asp-70041 isolates was minocycline (MICs, 0.25 mg/liter) followed by tigecycline (MICs, 0.5 mg/liter) and ciprofloxacin (MICs, 1 mg/liter) (11).

The ertapenem hydrolysis assay was performed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) using an extended period of incubation (4 h) as previously described (12). Both strains were detected as carbapenemase producers. The presence of carbapenemase-encoding genes was confirmed by PCR followed by DNA sequencing using specific primers (13–15), which demonstrated that both strains carried the carbapenem-hydrolyzing class D β-lactamase (CHDL)-encoding gene blaOXA-58. The clonal relatedness of the two OXA-58-producing A. seifertii isolates was evaluated by pulsed-field gel electrophoresis (PFGE) using the Apal restriction enzyme, as described previously (16). It revealed that Asp-1069 and Asp-70041 strains exhibited an indistinguishable PFGE pattern (data not shown). Southern blotting/hybridization experiments with a blaOXA-58-specific probe using the DIG DNA labeling and detection kit (Roche Diagnostics GmbH, Penzberg, Germany) were performed to determine the genetic location of blaOXA-58 after extraction of plasmid and genomic DNA, as previously published (17). Hybridization experiments showed that the blaOXA-58 gene was located on a plasmid of ~54 kb in both isolates.

Resistance to carbapenems among A. seifertii isolates has been reported in South Korea and Taiwan, associated with overexpression of efflux pumps or blaOXA-51-like carbapenemase mediated by ISAba1, respectively (7, 18). In addition, Marti and colleagues reported an OXA-58-producing A. seifertii isolate recovered from a Spanish hospital in the year 2000 (19). In that strain, blaOXA-58 was surrounded by two copies of ISAba3 and carried on a 100-kb plasmid. Interestingly, this isolate showed decreased susceptibility to imipenem (MIC, 6 mg/liter), differing from both isolates here described, which were highly resistant to imipenem and meropenem. Recently, Fu and colleagues reported plasmids of different sizes (52 to 143 kb) harboring the blaOXA-58 gene among Acinetobacter spp. in China (20). These authors reported that the blaOXA-58 genetic context was highly diverse and associated with different carbapenem susceptibility profiles. The resistance to carbapenems among A. seifertii isolates is very worrisome, since this species might naturally show a reduced susceptibility to polymyxins. Previous studies noticed a decreased susceptibility to polymyxins (MICs ranging from 2 to 64 mg/liter) among clinical and environmental A. seifertii isolates (7, 18, 21), which corresponded with our findings. Furthermore, the occurrence in both clinical and environmental specimens indicates that A. seifertii has a broad ecological niche, and acquisition of antibiotic genes in contaminated environments may lead to further accumulation of resistance genes in this potential pathogenic species.

To the best of our knowledge, Asp-1069 is the most ancient OXA-58-producing Acinetobacter strain reported to date (22). Although the blaOXA-58 gene was plasmid mediated in the A. seifertii isolates recovered at least 20 years ago, no further Acinetobacter isolates carrying this gene have been detected in our institution so
The studied strains were detected 4 years apart and belonged to the same PFGE clone; however, the clone source and reservoir remained undetermined. In fact, bla\textit{OXA-58} is rarely reported in Brazil (23), where \textit{bla}\textit{OXA-23} and \textit{bla}\textit{OXA-143} have been the most predominant CHDL genes (23, 24). The presence of carbapenemase-encoding genes in non-\textit{baumannii} species exhibiting decreased susceptibility to polymyxin is a cause of great concern, because it drastically limits the available therapeutic options for treatment of such infections.

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**REFERENCES**


**TABLE 1** Microbiological characterization of carbapenem-resistant \textit{A. seifertii} clinical isolates carrying \textit{bla}\textit{OXA-58}\textsuperscript{a}

<table>
<thead>
<tr>
<th>Strain</th>
<th>Species Source</th>
<th>Hospital unit</th>
<th>Date of isolation</th>
<th>MIC (mg/liter) of drug:</th>
</tr>
</thead>
<tbody>
<tr>
<td>A5-1069</td>
<td>\textit{A. seifertii} Tracheal aspirate</td>
<td>General ICU 10 September 1993</td>
<td>A</td>
<td>64/32 &gt;32 &gt;128 &gt;128 &gt;32 &gt;128 &gt;64 1 0.25 0.5 4</td>
</tr>
<tr>
<td>A5-70041</td>
<td>\textit{A. seifertii} Nasal secretion</td>
<td>Pneumology unit 3 November 1997</td>
<td>A</td>
<td>64/32 &gt;128 &gt;128 32 32 &gt;128 4 1 0.25 0.5 1</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Abbreviations: ICU, intensive care unit; PFGE, pulsed-field gel electrophoresis; SAM, ampicillin-sulbactam; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; IPM, imipenem; MEM, meropenem; AMK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; MIN, minocycline; TGC, tigecycline; PMB, polymyxin B.


