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 *bla*_{OXA-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 *bla*_{OXA-58}. The clonal relatedness of the two OXA-58-producing *A. seifertii* isolates was evaluated by pulsed-field gel electrophoresis (PFGE) using the *ApaI* 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 *bla*_{OXA-58}-specific probe using the DIG DNA labeling and detection kit (Roche Diagnostics GmbH, Penzberg, Germany) were performed to determine the genetic location of *bla*_{OXA-58} after extraction of plasmid and genomic DNA, as previously published (17). Hybridization experiments showed that the *bla*_{OXA-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 *bla*_{OXA-51}-like hyperexpression mediated by *ISAbal*, 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, *bla*_{OXA-58} was surrounded by two copies of *ISAbal* 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 *bla*_{OXA-58} gene among *Acinetobacter* spp. in China (20). These authors reported that the *bla*_{OXA-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 *bla*_{OXA-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

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

Strain	Species	Source	Hospital unit	Date of isolation	PFGE	MIC (mg/liter) of drug:										
						SAM	CAZ	CRO	FEP	IPM	MEM	AMK	GEN	CIP	MIN	TGC
Asp-1069	<i>A. seifertii</i>	Tracheal aspirate	General ICU	10 September 1993	A	64/32	>32	>128	>128	>32	>32	>128	>64	1	0.25	0.5
Asp-70041	<i>A. seifertii</i>	Nasal secretion	Pneumology unit	3 November 1997	A	64/32	>32	>128	32	32	32	>128	4	1	0.25	0.5

^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.

far. 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*_{OXA-58} is rarely reported in Brazil (23), where *bla*_{OXA-23} and *bla*_{OXA-143} have been the most predominant CHDL genes (23, 24). The presence of carbapenemase-encoding genes in non-*baumannii* species exhibiting decreased susceptibility to polymyxins is a cause of great concern, because it drastically limits the available therapeutic options for treatment of such infections.

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