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Antimicrobial Susceptibility Studies

Emergence of extensively drug-resistant OXA-72–producing *Acinetobacter baumannii* in Recife, Brazil: risk of clonal dissemination?

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ABSTRACT

Two new examples of OXA-72–producing *Acinetobacter baumannii* isolate resistant to a broad spectrum of antimicrobials, but not polymyxin B, have been identified in Recife, Brazil. Molecular typing indicated a close genetic link with the OXA-72–producing *A. baumannii* previously isolated in São Paulo, suggesting the possibility of clonal dissemination within the country.

Infections caused by multidrug-resistant *Acinetobacter baumannii* are extremely difficult to treat. Acquired carbapenem resistance in *A. baumannii* is frequently associated with Ambler class D carbapenemase production such as OXA-23, OXA-24, OXA-51, OXA-58, and OXA-143 groups or clusters (Zavascki et al., 2010). The intrinsic $bla_{\text{OXA-51}}$ gene is characteristic of this species and is usually weakly expressed (Zavascki et al., 2010). However, it can play a role in carbapenem resistance when an IS*Aba1* precedes the gene, providing promoter sequences that increase its expression (Werneck et al., 2011).

Outbreaks of OXA-producing *A. baumannii* have occurred throughout the world, including Brazil. However, these reports are restricted to the south and southeastern regions of the country, and until now, there have been no reported cases of the occurrence of this phenotype in the north east (Zavascki et al., 2010).

From a total of 228 *A. baumannii* isolates recovered from different patients admitted to 2 teaching hospitals in Recife, Brazil, from January 2010 to November 2011, 2 isolates that tested positive in the modified Hodge test and which had a XDR (extensively drug resistant) phenotype were selected. The first isolate (Ac 041) came from a tracheal aspirate sample taken from a 46-year-old man who had an AIDS diagnosis in 2010. The second isolate (Ac 928) came from a uroculture of a 60-year-old hypertensive, diabetic man with renal insufficiency and who had had a heart attack episode in 2011. Bacterial identification was carried out in accordance with standard biochemical tests and confirmed by means of the automated Mini API® System ID 32 E (bioMérieux, Marcy-l'Étoile, France). Susceptibility testing was performed by disk diffusion, Etest®, and the broth microdilution method, according to CLSI (2012) guidelines.

The isolates were screened for metallo-β-lactamase (MBL) production by the disk approximation test with 2-mercaptopropionic acid. The resulting phenotype from the MBL test was negative for both isolates. PCRs targeting $bla_{\text{SPM-1}}$, bla_{IMP}, and bla_{VIM} genes were carried out as previously described (Cavalcanti et al., 2012). None of the isolates showed amplification for MBL genes. A multiplex PCR assay targeting carbapenem-hydrolysing class D β-lactamase–encoding genes was performed (Higgins et al., 2010). The presence and position of IS*Aba1* and the presence of class 1 and 2 integrons were also investigated. The amplicons were purified with the aid of a PCR purification kit (Invitrogen, Carlsbad, CA, USA) and submitted for DNA sequencing. The nucleotide sequences were evaluated using BioEdit™ software (Ibis Biosciences, Carlsbad, USA) and submitted for online BLASTn analysis at Genebank (NCBI).

PCR experiment resulted in a 246-bp amplicon in both isolates that showed 100% identity with the $bla_{\text{OXA-72}}$ gene sequence deposited in the GenBank database. Plasmid extraction of the Ac 041 and Ac 928 isolates, followed by electrophoresis and subsequent PCR, showed that the $bla_{\text{OXA-72}}$ was located in plasmids of ~44 kb and ~11 kb, respectively (Table 1). Amplification product for the $bla_{\text{OXA-51}}$-like gene was also obtained. The PCR with combined primers targeting the $bla_{\text{OXA-51}}$-like or $bla_{\text{OXA-24}}$-like and the insertion sequence IS*Aba1* (IS*Aba1* forward/OXA-51-like reverse and IS*Aba1* forward/OXA-24-like reverse) showed negative results (Table 1), indicating that the expression of these genes was not driven by the promoter present in this insertion sequence element.

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Table 1
Characteristics of *Acinetobacter baumannii* isolates studied.

Strain	MBL test	OXA genes	Integrase genes	IS*Aba1*	IS*Aba1* + bla_{OXA-51}	IS*Aba1* + bla_{OXA-72}
Ac 041	neg	bla_{OXA-51}/ bla_{OXA-72}	*intI1*/*intI2*	+	neg	neg
Ac 928	neg	bla_{OXA-51}/ bla_{OXA-72}	*intI2*	+	neg	neg

Strain	IPM[a]	MEM[a]	CAZ[a]	ATM[a]	PMB[a]	CIP[b]	TZP[b]	GEN[b]
Ac 041	8	>32	>256	>64	0.38	>8	128	32
Ac 928	>32	>32	>256	96	0.19	>8	256	8

ATM = aztreonam; CAZ = ceftazidime; CIP = ciprofloxacin; GEN = gentamicin; IPM = imipenem; MEM = meropenem; PMB = polymyxin B; TZP = piperacilin/tazobactam; neg = negative; + = positive.
[a] Antimicrobial susceptibility testing by Etest®.
[b] Antimicrobial susceptibility testing by broth microdilution method.

The isolates were resistant to all of the antimicrobials tested except polymyxin B. This is compatible with an XDR phenotype (Table 1). A combination of different resistance mechanisms such as efflux pump overexpression, porin loss, penicillin-binding protein modification, and the presence of multiple gene cassettes carried by class 1 and 2 integrons should also be considered as possible factors determining this phenotype, contributing to its clinical severity (Migliavacca et al., 2013; Zavascki et al., 2010).

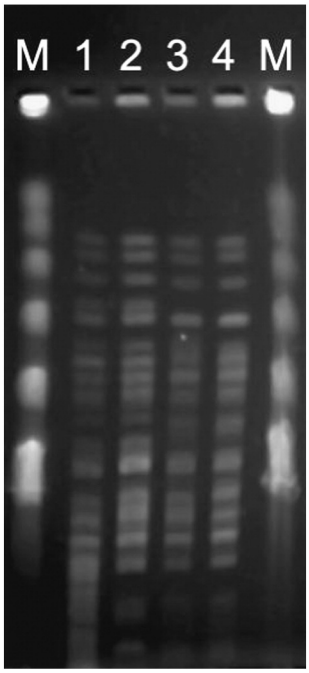

Fig. 1. PFGE of *Sma*I-digested chromosomal DNA of the OXA-72–producing *A. baumannii* isolates. M = lambda ladder PFG Marker (New England Biolabs); lanes 1 and 2 = isolates 041 and 928, respectively; lanes 3 and 4 = isolates from São Paulo (Werneck et al., 2011; Antonio et al., 2011, respectively). Isolate in lane 4 is representative of the 2 identical ERIC types detected by the authors.

Molecular typing using the pulsed-field gel electrophoresis (PFGE) technique (Fig. 1) revealed that the 2 isolates were indistinguishable from one another and closely related to 3 interrelated *A. baumannii* isolates that harbor the bla_{OXA-72} gene recently identified in São Paulo (Antonio et al., 2011; Werneck et al., 2011). This finding is indicative of the beginning of the spread of an epidemic clone across the country, as happened with the bla_{SPM-1} in *Pseudomonas aeruginosa* clone (Cavalcanti et al., 2012; Zavascki et al., 2010).

The OXA-24–like carbapenemases comprise OXA-40, OXA-25, OXA-26, and OXA-72 enzymes (Zavascki et al., 2010). The first enzymes of this group were originally described in isolates of *A. baumannii* from the Iberian Peninsula and, for a time, were restricted to Europe and USA, with no reports in Brazil until 2011 (Antonio et al., 2011; Werneck et al., 2011; Zavascki et al., 2010). OXA-72 was first described in an *Acinetobacter* isolate from Thailand in 2004 and has since been reported in a single *A. baumannii* strain from China and as a major mechanism of carbapenem resistance in a Taiwanese hospital (Werneck et al., 2011).

In conclusion, we report 2 cases of OXA-72–producing *A. baumannii* with an XDR profile identified in 2 different hospitals in Recife, which are strongly related to the isolates identified in São Paulo. This observation reinforces the continued importance of careful epidemiological surveillance and enhanced control measures in this country. The present work emphasizes the potential for this gene to spread between different countries and distinct geographical regions, thus further restricting the therapeutic options available to patients.

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