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SHORT REPORT
Carbapenem-susceptible *Acinetobacter baumannii* carrying the IS*Aba1* upstream $bla_{\text{OXA-51-like}}$ gene in Porto Alegre, southern Brazil

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SUMMARY

Over the last decade, *Acinetobacter baumannii* resistant to carbapenems has emerged in many medical centres and is commonly associated with high morbidity and mortality. We investigated potential mechanisms contributing to antimicrobial resistance of 58 clinical isolates of *A. baumannii* collected during a prolonged city-wide outbreak in five different hospitals in southern Brazil. The integrase gene was detected in 51 (87·9%) isolates of which 36 harboured class 2 integrons alone and 14 had both class 1 and 2 integrons; all carbapenem-resistant isolates displayed class 2 integrons. IS*Aba1* was found upstream of $bla_{\text{OXA-23-like}}$ only in isolates resistant to carbapenems; however, IS*Aba1* upstream of $bla_{\text{OXA-51-like}}$ was present in both susceptible and resistant isolates. This is the first report of a high prevalence of class 2 integrons in *A. baumannii* in southern Brazil. Moreover, our study suggests that IS*Aba1*/$bla_{\text{OXA-51-like}}$ alone is insufficient to confer resistance to carbapenems.

Key words: Antibiotic resistance, emerging infections, hospital-acquired (nosocomial) infections, infectious disease epidemiology, molecular epidemiology.

Acinetobacter baumannii is characterized by its tendency to acquire resistance to multiple classes of antimicrobial agents, including carbapenems [1]. This resistance has been linked to the presence of integrons and other genetic elements such as the insertion sequence IS*Aba1*. Integrons contain gene cassettes that carry resistance determinants and have been implicated in several outbreaks of opportunist species, particularly *A. baumannii*, in hospitals [2]. Five classes of integrons have been described based on the sequence of their *intI* genes, and class 1 is by far the most prevalent in *A. baumannii* isolates [2, 3]. The IS*Aba1* element has been found in association with carbapenem-resistance genes $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-58-like}}$ and AmpC cephalosporinase. IS*Aba1* appears to provide a promoter sequence which results in the overexpression of these resistance genes, as well as modulating the mobility of OXA-type genes [1, 4].

In early 2007, in Porto Alegre, southern Brazil, many hospitals simultaneously reported infections due to multidrug resistant *A. baumannii*. The increased infection rates persisted for at least 12 months and four intensive care units, in different hospitals,

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were closed due to this outbreak. A previous study of our group characterized 239 isolates of carbapenem-resistant (meropenem and/or imipenem) *A. baumannii* obtained from patients from five hospitals between July 2007 to June 2008 in Porto Alegre, and identified 14 distinct clonal groups by molecular techniques [5]. From that study we selected a total of 41 carbapenem-resistant isolates as representatives of each clonal group, including at least one isolate from the five hospitals, to evaluate the influence of mobile genetic elements in resistance to carbapenems. We also included 17 carbapenem-susceptible *A. baumannii* resulting in a total of 58 isolates. The isolates were identified using an automated system (Vitek, bioMérieux, France) and/or standard phenotypic methods performed in the clinical microbiology laboratory of each hospital and species-specific PCR for the $bla_{\text{OXA-51-like}}$ gene [6].

Minimal inhibitory concentrations (MICs) were determined by broth microdilution and/or the M.I.C.Evaluators™ method (Oxoid, UK) to the following antimicrobial agents: ceftazidime (Novafarma, Brazil), ampicillin/sulbactam (Eurofarma, Brazil), polymyxin B (Eurofarma), imipenem (ABL, Brazil) and meropenem (Eurofarma). Susceptibility results were interpreted according to Clinical and Laboratory Standards Institute guidelines [7]. *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used for quality control.

The presence of $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$ and $bla_{\text{OXA-143}}$ was detected by multiplex PCR assay using primers and conditions described previously [5, 8]. The isolates were screened for the presence of class 1 and class 2 integrons by PCR using primers to the integrase gene according to Koeleman *et al.* [9].

The insertion sequence IS*Aba1* upstream of $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-51-like}}$ was sought with primers IS*Aba1*F and OXA-23-likeR or OXA-51-likeR according to Turton *et al.* [4]. The detection of IS*Aba1*F/OXA-51-likeR was conducted in 25 μl volumes containing 1 × PCR buffer, 200 μM dNTPs, 1·0 U Top*Taq* DNA polymerase (Qiagen, Germany), 10 pmol of each primer and 3 μl bacterial lysate. The reaction mix for IS*Aba1*F/OXA23-likeR contained in 25 μl, 1 × PCR buffer, 200 μM dNTPs, 1·0 U Top*Taq* DNA polymerase (Qiagen), 7 pmol of each primer and 10 μl bacterial lysate. Cycling conditions for both PCR reactions were as described by Segal *et al.* [10].

IntI1, *IntI2*, IS*Aba1*/OXA-51 and IS*Aba1*/OXA-23 genes were sequenced on an ABI-PRISM 3100 Genetic Analyzer (ABI Ltd, USA). DNA sequences were analysed using the Basic Local Alignment Search Tool to search Genbank for homologous nucleic acid sequences.

Fisher's exact test was used to compare discrete variables. A P value $<0{\cdot}05$ was considered statistically significant. All analyses were performed with SPSS software, version 13.0 (IBM, USA).

All 41 carbapenem-resistant isolates presented a high level of resistance (MIC_{90} ampicillin/sulbactam, 64/32 μg/ml; MIC_{90} ceftazidime, >256 μg/ml; MIC_{90} polymyxin B, 4 μg/ml; MIC_{90} imipenem, >32 μg/ml; MIC_{90} meropenem, >32 μg/ml) to the five antibiotics tested in contrast to the 17 carbapenem-susceptible isolates which demonstrated susceptibility to ampicillin-sulbactam and polymyxin B (MIC_{90} ampicillin-sulbactam, 16/8 μg/ml; MIC_{90} polymyxin B, 1 μg/ml). Eight (13·8%) isolates showed resistance to polymyxin B ($\geqslant$4 μg/ml), including one isolate which was susceptible to carbapenems.

All isolates harboured the $bla_{\text{OXA-51-like}}$ gene, characteristic of *A. baumannii*. Thirty-nine (67·2%) carbapenem-resistant isolates and one carbapenem-susceptible isolate were positive for the $bla_{\text{OXA-23-like}}$ gene. Genes for $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$ or $bla_{\text{OXA-143}}$ were not detected in any isolate. IS*Aba1* was located upstream of the $bla_{\text{OXA-23-like}}$ gene in the majority (36/40) of isolates harbouring this gene. All isolates with this association displayed high MICs (MIC_{90} imipenem, $\geqslant$32 μg/ml; MIC_{90} meropenem, $\geqslant$32 μg/ml) for both carbapenems tested. In 23 (39·5%) isolates IS*Aba1* was located upstream of the $bla_{\text{OXA-51-like}}$ gene and 14 of these were also positive for IS*Aba1*/$bla_{\text{OXA-23-like}}$, with one isolate having the $bla_{\text{OXA-23-like}}$ gene alone. Therefore, eight isolates had the sole association of IS*Aba1*/$bla_{\text{OXA-51-like}}$ gene and these were susceptible to at least one of the carbapenems.

Integrase genes were detected in 51 (87·9%) of the 58 isolates analysed and were present in all of the carbapenem-resistant isolates and 10 (62·5%) of the carbapenem-susceptible isolates. Class 2 integrons were the most prevalent (86·2%), 14 (24·1%) isolates had both classes and a single isolate harboured only the class 1 integron. Each of the eight polymyxin-resistant isolates harboured both classes.

According to the molecular characterization of resistance determinants of each isolate, 12 groups were established (Table 1). Five groups (1, 2, 4, 5, 6) included solely carbapenem-susceptible isolates, five groups (8, 9, 10, 11, 12) included carbapenem-resistant

Table 1. *Molecular characterization of resistance determinants of carbapenem-resistant strains of* A. baumannii

Group (*n*)	*bla*$_{OXAs}$	*IntI1*	*IntI2*	IS*Aba1*/*OXA-23*	IS*Aba1*/*OXA-51*
1 (5)*	51	−	−	−	−
2 (2)*	51	−	−	−	+
3 (4)†	51	−	+	−	−
4 (5)*	51	−	+	−	+
5 (1)*	51	+	−	−	−
6 (1)*	51	+	+	−	+
7 (3)‡	23, 51	−	+	−	−
8 (1)§	23, 51	−	+	−	+
9 (16)§	23, 51	−	+	+	−
10 (7)§	23, 51	−	+	+	+
11 (6)§	23, 51	+	+	+	−
12 (7)§	23, 51	+	+	+	+

* All isolates of this group were susceptible to carbapenems.
† Two carbapenem-susceptible isolates and two carbapenem-resistant isolates.
‡ One carbapenem-susceptible isolate and two carbapenem-resistant isolates.
§ All isolates of this group were resistant to at least one carbapenem.

isolates only, and isolates from groups 3 and 7 showed variable susceptibility to carbapenems. Although susceptible isolates displayed IS*Aba1* upstream of *bla*$_{OXA\text{-}51\text{-}like}$, none of these presented this association with the *bla*$_{OXA\text{-}23\text{-}like}$ gene (Table 1).

Our study included isolates collected over a 1-year period from five major hospitals in Porto Alegre, Brazil. They were selected to be representative of distinct clonal groups, defined earlier by repetitive sequence PCR and pulsed-field gel electrophoresis [5], in order to evaluate the prevalence and dissemination of resistance determinants in different strain clusters. We found that in almost all carbapenem-resistant isolates the *bla*$_{OXA\text{-}23\text{-}like}$ gene was accompanied by the IS*Aba1* element. This was consistent with the premise that the degree of carbapenem resistance in these isolates was accentuated by the presence of promoter sequences provided by IS*Aba1*, leading to expression of the enzyme OXA-23, as previously demonstrated with European strains [4]. The carriage of IS*Aba1* upstream of *bla*$_{OXA\text{-}23\text{-}like}$ in almost all genetically unrelated isolates, obtained in different periods and from different hospitals, indicates that this genetic context is highly transmissible among different strains of carbapenem-resistant *A. baumannii*.

It is of note that nine (15·5%) isolates with IS*Aba1* upstream of *bla*$_{OXA\text{-}51\text{-}like}$ were susceptible to carbapenems. It might be expected that this combination should confer resistance to the carbapenems, as proposed by Turton *et al.* [4]. However, our data suggest that the promotion of the *bla*$_{OXA\text{-}51\text{-}like}$ without an efficient transcription of the gene is insufficient to confer resistance to carbapenems. These findings were corroborated in a study published by Bratu *et al.* [11] in which *A. baumannii* imipenem-susceptible isolates displayed the IS*Aba1*/*bla*$_{OXA\text{-}51\text{-}like}$ association. The lower transcript level of *bla*$_{OXA\text{-}51\text{-}like}$ might be due to a dysregulation of the transcription process, or to a disruption of the *bla*$_{OXA\text{-}51\text{-}like}$ gene, as already demonstrated [12]. This would justify the fact that, in this study, no statistically significant ($P=0{\cdot}36$) relationship between the presence of IS*Aba1* upstream of *bla*$_{OXA\text{-}51\text{-}like}$ and carbapenem resistance was found. Further studies on gene expression are required to evaluate fully the role of IS*Aba1* on transcription levels of *bla*$_{OXA\text{-}51\text{-}like}$ and *bla*$_{OXA\text{-}23\text{-}like}$ genes.

Class 1 integrons are distributed worldwide and are by far the most common in clinical isolates of Gram-negative bacteria, including the genus *Acinetobacter* [3]. Although class 2 integrons are rare in *Acinetobacter* species in USA, Europe and Asia [9], some studies have demonstrated a high prevalence of this element in Latin American countries, including Brazil [13, 14]. Our study corroborates these data and is the first to report this high prevalence in southern Brazil.

It is important to highlight that isolates belonging to groups 9, 10, 11 and 12 were disseminated among the five hospitals involved in the study and remained for a long period in the hospital setting as previously reported by our group [5]. The ubiquity of class 2 integrons in all carbapenem-resistant isolates underlines their propensity to harbour resistance gene cassettes which can spread and persist in strains of *A. baumannii* in the hospital environment.

DECLARATION OF INTEREST

None.

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