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SHORT REPORT
Widespread dispersion of the resistance element *tet*(B)::IS*CR2* in XDR *Acinetobacter baumannii* isolates

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SUMMARY

Acinetobacter baumannii is a significant nosocomial pathogen often associated with extreme drug resistance (XDR). In Argentina, isolates of *A. baumannii* resistant to tetracyclines have accounted for more than 40% of drug-resistant isolates in some hospitals. We have previously reported the dispersion of the *tet*(B) resistance element associated with the IS*CR2* transposase in epidemiologically unrelated *A. baumannii* isolates recovered from 1983 to 2011. This study extends this surveillance to 77 recent (2009–2013) XDR *A. baumannii* isolates with different levels of minocycline susceptibility. Isolates were examined by a pan-PCR assay, which showed six different amplification patterns, and specific PCRs were used for the confirmation of the the ΔIS*CR2*-*tet*(B)-*tet*(R)-IS*CR2* element. The *tet*(B) gene was present in 66 isolates and the IS*CR2* element in 68 isolates; the *tet*(B) gene was associated with IS*CR2* in all *tet*(B)-positive isolates. We conclude that this element is widespread in XDR *A. baumannii* isolates from Argentina and could be responsible for the emergence of tetracycline resistance in recent years.

Key words: Antibiotic resistance, genetics, microbiology.

Acinetobacter baumannii is widely recognized as a significant extreme drug-resistant (XDR) nosocomial pathogen [1], which has an intrinsic ability to develop resistance to several classes of antibiotics used to treat such infections. Specifically, resistance to carbapenem agents has increased markedly in recent years and in some countries, such as Argentina, accounts for almost 85% of clinical isolates of the species (http://antimicrobianos.com.ar/2013/10/informe-resistencia-2012-argentina/). In Argentina we have witnessed the emergence of minocycline resistance in *A. baumannii* reaching 40% of isolates in some centres [2] but there are few reports on the level of resistance in other countries with the exception of Iran where 17% of *A. baumannii* hospital isolates exhibited resistance to this agent [3].

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There are few options for the treatment of XDR *A. baumannii* infections but some studies have shown that combining the tetracycline, tigecycline, with colistin can be efficacious for such cases [4]. Furthermore, recent evidence indicates that minocycline offers an alternative treatment option for minocycline-susceptible, but otherwise multiply resistant *A. baumannii* strains when used in combination with colistin and/or carbapenems [5], leading to the proposal that intravenous treatment of multiply resistant *A. baumannii* pneumonia with minocycline offers a viable treatment option for such patients [6].

Given this clinical validation of the use of tetracyclines in these infections it is important to survey the incidence of resistance to these agents in *A. baumannii* and document and characterize the genetic elements that confer resistance in clinical isolates. Recently, our group has reported the dispersion of tetracycline resistance determinants in 47 epidemiologically unrelated *A. baumannii* isolates in Argentina and identified the presence of the *tet*(B) gene in about one-third of these isolates [7]. This was substantiated the finding of Nigro & Hall [8] that *tet*(B) was associated with the IS*CR2* transposase (both, the full and truncated version of the gene) in a representative isolate of the carbapenem-resistant global international clone II, which is suggestive of a possible dispersion of the clone. In this paper we present the results of a molecular surveillance study to map the dispersion of the ΔIS*CR2*-*tet*(B)-*tet*(R)-IS*CR2* element in recent XDR *A. baumannii* isolates recovered in recent years in Argentina.

Seventy-seven epidemiologically unrelated XDR *A. baumannii* isolates recovered from six hospitals in different regions of Argentina during 2009–2013 were studied (Table 1). The isolates were recovered from a variety of clinical sites and samples from individual patients, e.g. blood, urine, and respiratory tract. Isolates were confirmed as *A. baumannii* by MALDI–TOF MS (Bruker Daltonik, Germany) and *rpoB* amplification and sequencing. The resistance profiles of the isolates to ampicillin/sulbactam, piperacillin/tazobactam, cefepime, ceftazidime, imipenem, meropenem, ertapenem, gentamicin, amikacin, ciprofloxacin, levofloxacin, minocycline, tetracycline, trimethoprim-sulfamethoxazole, nalidixic acid, nitrofurantoin and colistin were determined by the disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines or by using the Vitek 2 System (bioMérieux, France) employing the panel AST-082 (GNS susceptibility card), and the minimum inhibitory concentration results were interpreted using the CLSI categories [9].

Total DNA was extracted according to the manufacturer's instructions (Wizard Genomic DNA Purification kit; Promega, USA) and subjected to amplification in a pan-polymerase chain reaction (PCR) assay of six genes to define the genetic relatedness of the isolates [10]. Those isolates showing a novel pattern in the pan-PCR assay that differed from known patterns of *A. baumannii* of the clonal complex (CC) circulating in Argentina, were subjected to multilocus sequence typing (MLST) according to the scheme of Bartual *et al.* [11] and allele sequences were compared at http://pubmlst.org/abaumannii/. The e-BURSTalgorithm (http://eburst.122mlst.net/) was used to assess the genetic relationship of sequence types (ST).

PCR reactions to amplify *tet*(B) and IS*CR2* genes, and to define the genetic context of *tet*(B) were performed using previously described specific primers [7]. To identify the presence of the AbaR-type element integrated within *comM*, which is the most common insertion site for this type of island, or *phoS*, which has been described as a secondary insertion site, four different PCR reactions (4 F/4R, 2 F/2R, 4R/2 F, phoF/phoR) were performed using previously described primers [12, 13]. These reactions additionally determined the location of ΔIS*CR2*-*tet*(B)-*tet*(R)-IS*CR2* within the AbaR-type genomic island. PCR amplification products were sequenced on both DNA strands using an ABIPrism 3100 BioAnalyzer and Taq FS Terminator Chemistry (PerkinElmer, USA) and analysed with Sequencher 4·7 software (Gene Codes Corp., USA) and BLAST v. 2·0 software (http://www.ncbi.nlm.nih.gov/BLAST/).

All isolates were categorized as XDR according to Magiorakos *et al.* [12], being resistant to carbapenems and all antibiotics tested except colistin, and in some cases to minocycline. All isolates were tetracycline resistant and 34 (44·1%) were fully resistant to minocycline, 30 (38·9%) were intermediately resistant, and three were susceptible to this antibiotic.

Six different amplification patterns were revealed by the pan-PCR. The amplification pattern corresponding to $CC113^{B}$, previously the most prevalent CC in our region, was observed in only two isolates (1 008 838 and 41 384). However, 33 (42%) isolates gave a pattern corresponding to $CC110^{B}$ indicating the emergence of this CC and a displacement of $CC113^{B}$ in the region. Nine isolates were assigned to $CC109^{B}$ (international clone 1), Furthermore, two novel pan-PCR patterns were identified which did not correspond to any of the patterns previously described in our region [13].

Table 1. *Description of the studied isolates and the corresponding PCR results obtained*

Isolate	Hospital*	Year	CC†	Minocycline susceptibility	IS*CR2*	*tet*(B)
59	H1	2012	CC110	S	+	+
65 948, 66 295	H1	2012	CC110	S	–	–
63, 97, 99, 154, 177, 180, 183, 204	H1	2012	ST1126	I	+	+
109	H1	2012	CC109	S	–	–
142	H1	2012	ST1126	S	+	+
156, 166, 210	H1	2012	CC110	I	+	+
178, 66 492	H1	2012	CC110	R	+	+
195	H1	2012	ST1126	I	+	–
194	H1	2012	ST1126	R	+	+
1557	H1	2013	CC110	R	+	–
M2	H1	2013	CC109	S	–	–
M16	H1	2013	CC110	R	+	+
980 417	H2	2012	CC110	I	+	+
1 008 838	H2	2012	CC113	R	+	+
1 015 804	H2	2012	ST1126	R	+	+
1 049 158	H2	2013	ST1126	I	+	+
1 049 648	H2	2013	CC110	I	+	+
38 471	H3	2013	CC110	I	+	+
41 384	H3	2013	CC113	I	–	–
112, 389	H4	2011	CC109	R	+	+
77, 84, 408, 416	H4	2011	CC110	I	+	+
164	H4	2011	CC110	R	+	+
424	H4	2011	CC110	I	–	–
104, 376, 527	H4	2011	ST1126	R	+	+
377, 379, 403, 414, 415, 423	H4	2011	ST1126	I	+	+
535	H4	2012	CC109	I	–	–
550	H4	2012	CC110	I	+	+
551	H4	2012	CC109	S	+	+
591	H4	2012	CC110	R	+	+
11 498	H5	2009	ST1126	R	+	+
11 537	H5	2009	CC110	R	+	+
11 634, 11 686, 11 710, 11 799, 11 822, 11 917, 11 948, 13 103	H5	2010	CC110	R	+	+
11 635, 11 804, 11 806, 13 232	H5	2010	ST1126	R	+	+
11 813	H5	2010	ST1125	R	+	+
13 427, 13 546	H5	2011	CC110	R	+	+
13 946	H5	2012	CC109	R	+	+
13 948	H5	2012	ST1125	R	+	+
13 956	H5	2012	CC103	R	+	+
1776, 2793	H6	2012	CC109	S	–	–
2080, 2081, 2718	H6	2012	ST1126	S	+	+
2749	H6	2012	CC110	S	+	+

* Location of the different hospitals in the Buenos Aires city neighbourhood: H1, Barracas; H2, Montserrat; H3, Constitución; H4, Villa Crespo; H5, Constitución; H6, Recoleta.

† Clonal complexes (CCs) were defined according to pan-PCR [10] and the Bartual scheme. MLST was used to confirm pan-PCR results of the new ST1125 and ST1126 [11].

One of the new patterns, first designated as P133, was found in 30 isolates (38%) indicating its wide distribution in the hospital setting. The other novel pattern, designated PM1, was present in only two isolates (11 813and 13 948), and is identical to the pattern described from the USA for the strain Ab04 (E. Snitkin, personal communication). A single isolate (13 956) gave a pattern consistent with the control strain of CC103^{B}.

Isolates displaying the novel patterns P133 and PM1 were typed by MLST. This confirmed that they corresponded to novel STs with the allelic profiles of 1–15–2–2–91–262–31 for P133 isolates (assigned ST1126), and 1–3–3–2–90–7–3 for PM1 isolates

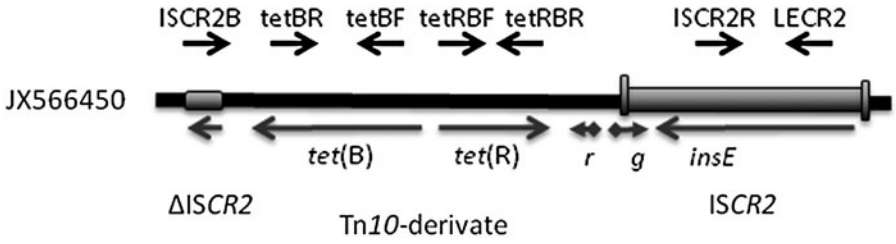

Fig. 1. Schematic representation of *tet*(B)::IS*CR2* genetic element. Boxes and lines of different thickness and colour represent determinant and antibiotic resistance elements. Vertical bars indicate the inverted repeats of the IS*CR2* element. ΔIS*CR2* is a partial version of the IS*CR2* element. Genes are shown by horizontal arrows; phosphoglucosamine mutase (*glmM*) and the transcription regulator of ArsR family genes are represented as g and r, respectively. Primers are indicated by horizontal black arrows. The *tet*(B)-IS*CR2* element is available from GenBank under accession number ANJX566450.

(assigned ST112). ST1126 could not be grouped by the eBURST algorithm within an existing CC but ST1125 was grouped as a single locus variant of ST92 within international clone II.

The *tet*(B) gene was present in 66 (86%) of the 77 *A. baumannii* isolates tested and IS*CR2* was found in 68 isolates; all *tet*(B)-positive isolates harboured the complete IS*CR2* element (Table 1). We have previously described the presence of a truncated IS*CR2* (ΔIS*CR2*) downstream of the *tet*(B) gene [8]. In this study PCR amplification with specific primers (ISCR2B/tetBF) for ΔIS*CR2* confirmed that all *tet*(B)-*tet*(R)-IS*CR2*-positive isolates ($n = 66$), where IS*CR2* is upstream of the *tet*(B) gene, contain ΔIS*CR2* downstream of *tet*(B), supporting the wide dispersion of ΔIS*CR2*-*tet*(B)-*tet*(R)-IS*CR2* in our *A. baumannii* population (Fig. 1). BLAST analysis against GenBank database sequences, also revealed this element in four other *A. baumannii* isolates, one of them being Ab 13 205 previously identified by our group [7]; the other three isolates (ZW85–1, BJAB0715, A91) shared 99% identity with the ΔIS*CR2*-*tet*(B)-*tet*(R)-IS*CR2* sequence (GenBank accession no. JX566450; Fig. 1). In one of the latter isolates the element was located in a plasmid, ZW85p2, and in the other two strains the element was chromosomally located within the AbaR-type genomic island [8]. As our earlier work did not find *tet*(B) within the AbaR-type genomic organization [7], we sought to verify the location of *tet*(B) using specific PCR reactions (4 F/4R, 2 F/2R, 4R/2 F, phoF/phoR) for the intact *comM* and *phoS* genes [13, 14] and confirmed that this gene was intact in all the studied isolates.

This study presents data supporting an increase in the dispersion of the ΔIS*CR2*-*tet*(B)-*tet*(R)-IS*CR2* resistance element in XDR *A. baumannii* isolates recently recovered in Argentina, compared to our earlier survey [7]. This increase (86% *vs.* 28%) explains in part the observed rise in minocycline resistance in recent years. Minocycline, together with colistin, has been reported to be the only antimicrobials to which at least 50% of clinical *A. baumannii* isolates are susceptible and are therefore drugs of last resort for the treatment of such infections [5]. Moreover, a recent review of extensive data supports the successful use of minocycline for MDR strains [6]. An increase in minocycline resistance should therefore act as a stimulus in consideration of new combinations of agents such as tigecycline with colistin, or doxycycline with amikacin as alternative therapies, particularly for severe infections caused by XDR strains of the species.

In conclusion, our data taken in combination with our earlier study [7] add weight to the conclusion that within the resistance element ΔIS*CR2*-*tet*(B)-*tet*(R)-*ISCR2*, the linkage of the *tet*(B) gene with the insertion sequence IS*CR2*, provides a genetic mechanism for resistance that has spread in *A. baumannii* isolates in Argentina. In addition, we have documented the emergence of a new lineage (ST1126) and the possible displacement of the clonal complex CC113 by CC110, which is indicative of a genetically dynamic *A. baumannii* population. Last, we report the first identification of isolates belonging to international clone II in Argentina which had hitherto only been documented in South America in Curitiba, Brazil [15]. These data highlight the importance of performing molecular genetic surveillance studies to identify and monitor rate changes in the antimicrobial resistance determinants in *A. baumannii* populations.

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DECLARATION OF INTEREST

None.

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