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Emergence and Spread of Plasmid-Borne *tet*(B)::IS*CR2* in Minocycline-Resistant *Acinetobacter baumannii* Isolates

Elisabet Vilacoba,[a] Marisa Almuzara,[b] Lucia Gulone,[a] German Matías Traglia,[a] Silvia A. Figueroa,[b] Gabriela Sly,[b] Analia Fernández,[c] Daniela Centrón,[a] María Soledad Ramírez[a]

Instituto de Microbiología y Parasitología Médica (IMPaM, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires,[a] Laboratorio de Bacteriología, Hospital Interzonal de Agudos Eva Peron, San Martin,[b] and Laboratorio de Microbiología, Hospital Universitario Fundación Favaloro,[c] Buenos Aires, Argentina

Resistance to minocycline has emerged in multidrug-resistant *Acinetobacter baumannii* isolates from Buenos Aires hospitals. Few reports about the description and dispersion of *tet* genes in this species have been published. We observed the presence of *tet*(B) in all minocycline-resistant isolates. This gene was found to be associated with the IS*CR2* mobile element, which may, in part, explain its dispersion.

Recently, misconceptions regarding *Acinetobacter baumannii* have been advanced, exposing the importance of this species as a significant nosocomial pathogen (1–3). One of the important features of *A. baumannii*, which may contribute to its success in surviving in the hospital environment, is the intrinsic ability to develop resistance to all available antibiotics to treat *A. baumannii* infections (1–3).

A review of the existing literature indicates that little is described regarding the dispersion of tetracycline resistance determinants (*tet*) among *A. baumannii* clinical isolates (4–8). The presence of resistance genes such as *tet*(A) and *tet*(B) for tetracyclines, most of them tetracycline efflux pumps, has been described (4–12). It is known that Tet(A) confers resistance to tetracycline (TET) while Tet(B) confers resistance to TET and minocycline (MIN). In Argentina, the emergence of minocycline resistance has been observed in the past few years, varying from 10 to 40% resistance among different centers (13).

As there are scarce data about the resistance determinants for tetracyclines in *A. baumannii* and the potential therapeutic benefit of using tetracyclines in combination treatment with colistin and carbapenems (14, 15), we decided to search for the presence of tetracycline resistance genes in 47 epidemiologically unrelated *A. baumannii* isolates. The studied isolates were selected from a collection of 250 *A. baumannii* clinical isolates. Criteria for selection included temporal representation (1983 to 2011), different hospitals ($n = 11$), and different countries (Argentina, Uruguay, and Chile). The selected isolates possessed different antibiotic resistance profiles, with all of them resistant to tetracycline and 12 also resistant to minocycline (Table 1). Using molecular typing techniques, we observed the presence of a prevalent clone among the minocycline-resistant isolates (Table 1).

To determine the presence of *tet* genes, we extracted total DNA and used it to perform PCR amplification reactions according to the manufacturer's instructions (Promega, Madison, WI). Specific primers for *tet*(A), *tet*(B), *tet*(M), *tet*(39), and *tet*(H) were used (see Table S1 in the supplemental material). We obtained positive results for the *tet*(A) and *tet*(B) genes, and the rest of the amplification reactions were negative (Table 1). All PCR amplification products were sequenced on both DNA strands using an ABI Prism 3100 BioAnalyzer and *Taq* FS Terminator Chemistry (*Taq* FS; Perkin-Elmer), and sequence analysis was performed with the Sequencher 4.7 software (Gene Codes Corp.) and BLAST (version 2.0) software (http://www.ncbi.nlm.nih.gov/BLAST/).

The *tet*(B) gene was found in 13 extensively drug-resistant *A. baumannii* isolates of our collection, with 12 of them minocycline resistant (Table 1). All positive isolates were recovered in the last year, exposing the recent emergence of this determinant (Table 1). There is scant information about *tet*(B) gene distribution among *A. baumannii* clinical isolates. The few reports which investigated the presence of this gene in *A. baumannii* clinical isolates showed a high prevalence of *tet*(B) among multidrug-resistant *A. baumannii* isolates (6, 12). Lately, the description of an AbaR-type genomic island in strains belonging to the global clone 2 revealed the presence of a *tet*(B) determinant in this context (9–11).

To determine the genetic platform of *tet*(B) in our isolates, inverse PCR and PCRs with primers annealing in the described structures were performed. Sequence analysis of the positive inverse PCR products revealed the presence of the IS*CR2* element downstream of *tet*(R) and a sequence derived from Tn*10*, which is preceded by the ΔIS*CR2* deletion, upstream of *tet*(B) (Fig. 1). The genetic platform surrounding *tet*(R) resembled the recently described genetic context found in the AbaR4-type islands (9–11). As Tn*5393*, containing the *strA* and *strB* genes, was found following the IS*CR2* element in the recently described *tet*(B) genetic platform (9–11), PCRs were carried out to determine their presence. All *tet*(B)-positive isolates contained the *strA* and *strB* genes associated with the IS*CR2* mobile element.

Concerning the structure upstream of the *tet*(B) gene, Nigro

Received 23 August 2012 Returned for modification 25 October 2012
Accepted 3 November 2012
Published ahead of print 12 November 2012
Address correspondence to María Soledad Ramírez, ramirez.mariasoledad@gmail.com.
Supplemental material for this article may be found at http://dx.doi.org/10.1128/AAC.01751-12.

doi:10.1128/AAC.01751-12

TABLE 1 Description of the isolates included in the study and PCR amplification results of *tet*(A) and *tet*(B)

Isolate	Hospital/country	Yr	Clone	Tetracycline resistance	*tet*(A)	*tet*(B)
Ab102	H7/Argentina	1983	II	TET	−	−
Ab155	H7/Argentina	1994	IV	TET	+	−
Ab115	H7/Argentina	1994	IV	TET	+	−
Ab1	H2/Chile	1997	III	TET	−	−
Ab3	H2/Chile	1997	III	TET	−	−
Ab6	H2/Chile	1998	III	TET	−	−
Ab181	H7/Argentina	2001	I	TET	−	−
Ab7	H3/Chile	2006	III	TET	−	−
Ab287	H4/Argentina	2006	XIV	TET	+	−
Ab374	H4/Argentina	2007	IV	TET	−	−
Ab326	H4/Argentina	2007	XIV	TET	−	−
Ab375	H5/Argentina	2007	I	TET	−	−
Ab394	H4/Argentina	2007	I	TET	−	−
Ab49631	H6/Argentina	2008	III	TET	−	−
Abu1	H1/Uruguay	2008	IV	TET	−	−
Abu2	H1/Uruguay	2008	IV	TET	−	−
Ab66285	H6/Argentina	2009	XIV	TET	+	−
Ab98	H8/Argentina	2009	III	TET	−	−
Ab04	H11/Argentina	2010	III	TET	−	−
Ab21	H10/Argentina	2010	III	TET	−	−
Ab908	H8/Argentina	2011	III	TET	−	−
Ab13205	H8/Argentina	2011	V	TET, MIN	−	+
Ab13338	H8/Argentina	2011	III	TET	−	−
Ab13494	H8/Argentina	2011	III	TET	−	−
Ab14393	H8/Argentina	2011	V	TET, MIN	−	+
Ab15111	H8/Argentina	2011	III	TET	−	−
Ab47	H8/Argentina	2011	III	TET	−	−
Ab15424	H8/Argentina	2011	III	TET	+	−
Ab15975	H8/Argentina	2011	III	TET	−	−
Ab15799	H8/Argentina	2011	III	TET	+	−
Ab15897	H8/Argentina	2011	III	TET	+	−
Ab15	H8/Argentina	2011	V	TET, MIN	−	+
Ab20	H8/Argentina	2011	V	TET, MIN	−	+
Ab42	H8/Argentina	2011	III	TET	+	−
Ab53	H8/Argentina	2011	III	TET	−	−
Ab103	H8/Argentina	2011	III	TET	−	−
Ab105	H8/Argentina	2011	V	TET, MIN	−	+
Ab106	H8/Argentina	2011	V	TET, MIN	−	+
Ab107	H8/Argentina	2011	V	TET, MIN	−	+
Ab112	H8/Argentina	2011	III	TET	−	−
Ab120	H8/Argentina	2011	III	TET	−	−
Ab129	H8/Argentina	2011	V	TET, MIN	−	+
Ab133	H8/Argentina	2011	V	TET, MIN	−	+
Ab741019	H9/Argentina	2011	V	TET, MIN	−	+
Ab761457	H9/Argentina	2011	V	TET, MIN	−	+
Ab767339	H9/Argentina	2011	V	TET, MIN	−	+
Ab795672	H9/Argentina	2011	VI	TET	−	+

and Hall (10) recently found this gene within a novel AbaR4-type island, named Tn*6167* and containing the *sul2* gene and a partial copy of CR2, which is the same genetic context identified in all *tet*(B)-positive isolates from our study. To determine that *tet*(B) was within the AbaR-type genomic organization, specific PCRs showing that the *comM* gene was intact were done (16). This gene was recognized as a target for the insertion of AbaR-type resistance islands in multidrug-resistant strains enclosing different resistance determinants (10, 16–18). Therefore, the difference concerning the *tet*(B) genetic platform is that our isolates do not have the *tet*(B) gene linked to AbaR islands (Fig. 1).

Plasmidic DNA from Ab13205 was extracted using the QIAfilter midi kit (Qiagen) and used to transform *Escherichia coli* Top10 competent cells. Colonies were obtained in plates containing 3 μg/ml of TET and subjected to plasmid DNA extraction as described above (data not shown). To confirm *tet*(B) presence, plasmidic DNA was used as the template for PCR amplification, obtaining positive results. Moreover, the MICs to TET and MIN of the transformed *E. coli* Top10 cells were determined using the Etest strips as recommended (19). MIC results show an increase in both MICs, supporting the plasmid location (Table 2).

On the other hand, we observed the presence of the *tet*(A)

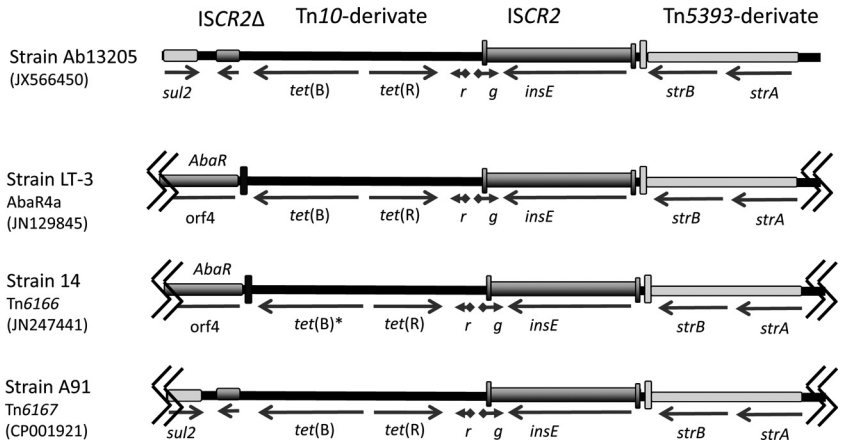

FIG 1 Schematic representation of the tet(*B*) gene context found in this study and the recently described context found in the AbaR4 islands. Boxes and lines of different thicknesses and colors represent the different determinants and elements (antibiotic resistance genes and IS*CR2*). Vertical bars indicate the inverted repeats and the *ori* and *ter* sites of the IS*CR2* element. IS*CR2*Δ is a partial version of the IS*CR2* element, and *tet*(B)* is a truncated version of the *tet*(B) gene. Genes are shown by horizontal arrows with the gene name listed below. The *tet*(B) genetic context found in Ab13205 resembles the recently described *tet*(B) context within the AbaR island found in strain A91 (10). Phosphoglucosamine mutase (*glmM*) and the transcription regulator of ArsR family genes are represented as g and r, respectively. The structure found in the study is available from GenBank under accession number JX566450. Information in parentheses after each strain name indicates the GenBank accession number for that strain.

gene in 8 out of 47 isolates. These *tet*(A)-positive isolates were recovered in different years (1994, 2006, and 2011) and different hospitals (Table 1). This gene was reported to be associated with Tn*1721*-like transposon, which was reported in a clinical *A. baumannii* isolate (7). In order to determine if this genetic structure was present in our isolates, PCRs with specific primers for the Tn*1721*-like transposase (*tnpA*) and *tet*(A) gene were carried out (see Table S1 in the supplemental material). When we tested the primer combinations tetAbTnpA′R/tetAF and tetAbTnpAF/tetAR, negative results were obtained in all *tet*(A)-positive isolates, exposing the absence of this transposon in our isolates and suggesting a different genetic context for this gene.

Our results exposed a complex genetic background associated with tetracycline resistance in our *A. baumannii* population, including *tet*(A), *tet*(B), and yet-unknown elements. We also observed the presence of the *tet*(B) gene associated with the plasmid-mediated IS*CR2* mobile element. Lastly, we noted that this element can be transferred and increased the MICs to tetracycline and minocycline in *E. coli*. This new genetic platform of the *tet*(B) gene, linked to the insertion sequence IS*CR2*, supports a mechanism by which spread among *A. baumannii* isolates is possible.

Nucleotide sequence accession number. The sequence of the *tet*(B) context has been submitted to GenBank under accession no. JX566450.

TABLE 2 MICs to TET and MIN[a]

Strain	MIC (mg/liter)	
	TET	MIN
A. baumannii Ab13205	32	12
E. coli TOP10	1	0.5
E. coli TOP10 p13205	24	3

[a] The MICs to TET and MIN were determined by following CLSI recommendations.

ACKNOWLEDGMENTS

M.S.R. and D.C. are career investigators of CONICET, Argentina. E.V. and G.M.T. have doctoral fellowships from CONICET. This study was supported by grant PIP 11420100100152 to M.S.R. and grant UBACyTs 2011-2012 to M.S.R. and D.C., Buenos Aires, Argentina.

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