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OXA-253, a Variant of the Carbapenem-Hydrolyzing Class D β-Lactamase OXA-143 in *Acinetobacter baumannii*

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The carbapenem-hydrolyzing class D β-lactamase OXA-253 was identified in an *Acinetobacter baumannii* clinical isolate belonging to sequence type 113 (ST113) in Brazil. OXA-253 shares 93.8% amino acid identity with OXA-143. The $bla_{OXA-253}$ gene is located on a ca. 20-kb plasmid. The genetic environment of the $bla_{OXA-253}$ gene shares the highest identity with ubiquitous GR2 group plasmids usually carrying $bla_{OXA-24/-40}$ genes.

Acinetobacter baumannii strains are frequently associated with nosocomial infections, and their resistance to carbapenems has risen in recent decades (1, 2). The main mechanisms of resistance to carbapenems of *A. baumannii* are efflux pumps, porin mutations, overexpression of the chromosomally encoded OXA-51-like β-lactamase, and the biosynthesis of acquired carbapenem-hydrolyzing class D β-lactamases (CHDLs) (oxacillinases) (3). To date, five main groups of CHDLs have been identified in *A. baumannii*, the intrinsic chromosomally encoded OXA-51-like enzyme and the acquired chromosomally encoded and plasmid-encoded OXA-23-like, OXA-40-like, OXA-58-like, and OXA-143-like enzymes (3). OXA-143 was first described in 2009 from an *A. baumannii* strain isolated in Brazil in 2004 (2). In this study, we report an *A. baumannii* clinical strain producing OXA-253, a variant of OXA-143. The genetic environment of the plasmid-borne $bla_{OXA-253}$ gene is detailed.

The clinical isolate *A. baumannii* 25 was recovered from a perineal swab in the intensive care unit in Minas Gerais Hospital, Brazil. MICs were determined by Etest (bioMérieux, La Balmes-Grottes, France). Isolate 25 was resistant to all β-lactams, including carbapenems, and had reduced susceptibility to cefepime according to the CLSI guidelines (4) (Table 1). It was also resistant to fluoroquinolones (ciprofloxacin), and susceptible to aminoglycosides (gentamicin, tobramycin, amikacin, and netilmicin), and tetracycline. A modified version of the CarbaNP test (5) and the CarbAcinetoNP test (P. Nordmann, personal communication) was performed and showed a carbapenemase activity. PCR experiments, performed as previously described for screening of bla_{OXA-23}, bla_{OXA-40}, bla_{OXA-58} and $bla_{OXA-143}$ genes (6), identified a $bla_{OXA-143}$-like gene. Further sequencing of the PCR product identified the $bla_{OXA-253}$ gene (GenBank number KC479324) and showed 95% nucleotide identity with the $bla_{OXA-143}$ gene. The deduced protein sequence showed 17 amino acid differences between OXA-143 and OXA-253 (93.8% identity) (Fig. 1). Those amino acid changes do not occur in the conserved residues STFK (position 70 to 73) and KSG (position 216 to 218) of class D β-lactamases or in the FGN structural element (position 144 to 146) of carbapenem-hydrolyzing class D β-lactamases (CHDLs) (Fig. 1).

Plasmid extraction from *A. baumannii* 25 performed by the Kieser technique yielded two plasmids of ca. 70 and 20 kb (7). The gene encoding the OXA-253 determinant from *A. baumannii* 25 was transferred to *Escherichia coli* TOP10 by electroporation. The resulting *E. coli* TOP10 strain harboring the plasmid of ca. 20 kb (pAb25) was selected on ticarcillin (50 μg/ml) containing Trypticase soy agar. Plasmid DNA from *E. coli* TOP10 (pAb25) was used as the template for sequencing the 3.59-kb sequence encompassing the $bla_{OXA-253}$ gene by primer walking, i.e., by using primers sequentially designed on the obtained sequence from the inside of the $bla_{OXA-253}$ gene to the outside. Surprisingly, the genetic environment of the $bla_{OXA-253}$ gene, with the open reading frames (ORFs) *tonB*, ORF2, ORF1, *repAci2*, and *repB*, differed greatly from that of the $bla_{OXA-143}$ gene described previously (2) but showed 95% identity with that of the $bla_{OXA-24/-40}$ gene of pABVA01 plasmid (GR2 group) from an *A. baumannii* strain from Italy (8), although the $bla_{OXA-253}$ gene shares only 88% nucleotide identity with the $bla_{OXA-24/-40}$ gene (Fig. 2). The $bla_{OXA-253}$ gene was most probably integrated via the XerC/XerD recognition site, located between the $bla_{OXA-253}$ gene and the replicase-coding gene *repAci2*, as recently described for other GR2 group plasmids carrying $bla_{OXA-24/-40}$-like genes in *A. baumannii* isolates (9) (Fig. 2). The integration of the $bla_{OXA-253}$ gene on this ubiquitous plasmid may facilitate the dissemination of this carbapenemase. In order to determine the role of OXA-253 in the carbapenem resistance of *A. baumannii* isolate 25, the natural plasmid harboring the $bla_{OXA-253}$ gene was electroporated into the *A. baumannii* reference strain CIP 7010, and MICs of β-lactams for the resulting strain *A. baumannii* CIP 7010(pOXA-253) were determined by Etest (Table 1). MICs of carbapenems were the same for *A. baumannii* CIP 7010(pOXA-253) and for *A. baumannii* 25, confirming that OXA-253 on its own was able to confer carbapenem resistance.

In order to compare the β-lactam resistance profile conferred by each oxacillinase, OXA-143 and OXA-253 were produced in the same genetic background. The PCR amplicon encompassing

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TABLE 1 MICs of β-lactams for strains tested

β-Lactam(s)	MIC (μg/ml)[a]					
	A. baumannii 25	*A. baumannii*(pOXA-253)	*A. baumannii* CIP7010	*E. coli* TOP10(pTOPO-OXA-253)	*E. coli* TOP10(pTOPO-OXA-143)	*E. coli* TOP10
Amoxicillin	>256	>256	32	>256	>256	4
Amoxicillin + CLA[b]	>256	>256	32	128	64	4
Ticarcillin	>256	>256	8	>256	128	4
Ticarcillin + CLA	>256	>256	8	128	64	4
Piperacillin	>256	>256	16	8	4	1
Ceftazidime	>256	4	4	0.38	0.38	0.06
Cefotaxime	>256	12	2	0.12	0.12	0.12
Cefepime	4	3	2	0.12	0.12	0.12
Aztreonam	>256	24	8	0.06	0.09	0.06
Imipenem	>32	32	0.25	1	0.38	0.06
Ertapenem	ND[c]	ND	ND	1	0.75	0.06
Meropenem	>32	>32	0.25	0.5	0.19	0.01

[a] The MICs were determined by Etest.
[b] CLA, clavulanic acid at a fixed concentration of 4 μg/ml.
[c] ND, not determinable due to the natural resistance of these Gram-negative rods to ertapenem.

the entire sequence of the $bla_{OXA-143}$ gene was obtained with primers OXA-143A (5′-TACCTTCGGACGTTTGAAAGTTC-3′) and OXA-143B (5′-TAGCTCCCAATTTCCGTTTGG-3′), and that of the $bla_{OXA-253}$ gene with primers OXA-253A (5′-AAGCCGACTT GTTTCAAAGTCGGC-3′) and OXA-253B (5′-ACTATTCGCAT GTTTAAGTGGCAC-3′). The corresponding genes were cloned in the same pTOPO vector (Qiagen, Courtaboeuf, France) under the same promoter and expressed in *E. coli* TOP10. MICs of the resulting strains *E. coli* TOP10(pTOPO-OXA-143) and *E. coli* TOP10(pTOPO-OXA-253) were determined by Etest. Comparison of MICs showed that OXA-253 conferred a higher level of resistance to carbapenems to *E. coli* TOP10 only than that conferred by OXA-143, with MICs being only 2-fold higher (Table 1).

Multilocus sequence typing was performed using specific primers and conditions described in the *A. baumannii* multilocus sequence type (MLST) database of the Pasteur Institute (www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html). MLST analysis showed that *A. baumannii* 25 belonged to ST113. Until now, this ST was not commonly recovered. Indeed, some *A. baumannii* isolates belonging to ST113 have been identified in Kuwait in 2013, containing plasmids harboring the bla_{GES-11} carbapenemase gene alone or coharboring the bla_{OXA-23} gene (10). More recently, several *A. baumannii* isolates belonging to ST113 or clonal complex 113 (CC113) (ST99 and ST227) were recovered from southeast Brazil, almost the same area as the hospital of Minas Gerais where this *A. baumannii* 25 isolate came from (11). Moreover, Clímaco et al. recovered the $bla_{OXA-143}$ gene from multiple STs of carbapenem-resistant *A. baumannii* strains (CRABs) (ST109, ST405, and ST406) (11), emphasizing that the dissemination of this carbapenemase gene is not related to a single clone.

CRABs have become increasingly isolated in Brazil, with those carrying bla_{OXA-23}-like and $bla_{OXA-143}$-like genes being most prevalent (11). As opposed to what occurs in the rest of the world, with the predominance of ST92/OXA-23 *A. baumannii* isolates, it seems that CRABs carrying bla_{OXA-23}-like and $bla_{OXA-143}$-like genes mainly belong to multiple STs and clonal complexes CC104, CC109, and CC113 in Latin American countries.

Nucleotide sequence accession number. The nucleotide sequence of the $bla_{OXA-253}$ gene is available under GenBank accession number KF824909.

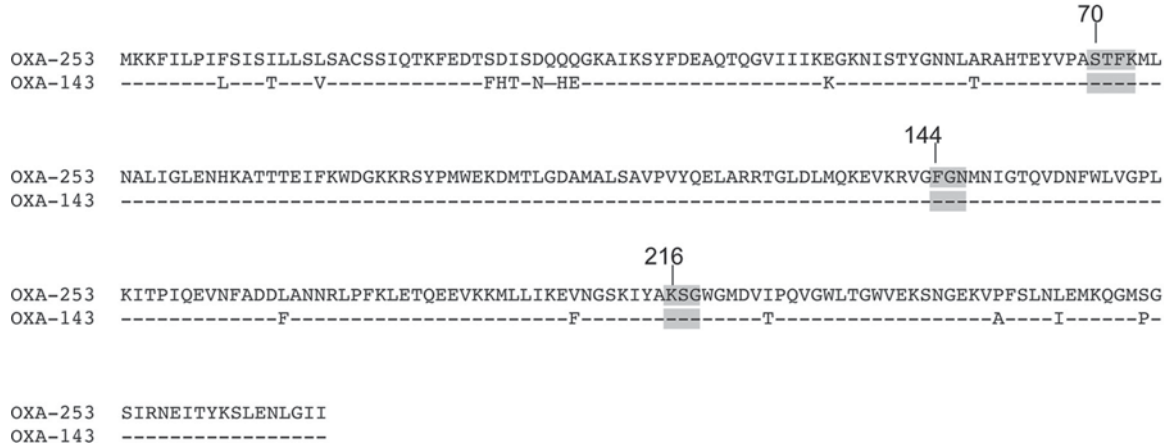

FIG 1 Alignment of the OXA-253 amino acid sequence with that of OXA-143. Conserved residues among class D β-lactamases (DBLs) are shaded. Dashes represent conserved amino acids. β-Lactamases are numbered according to the DBL numbering system (12).

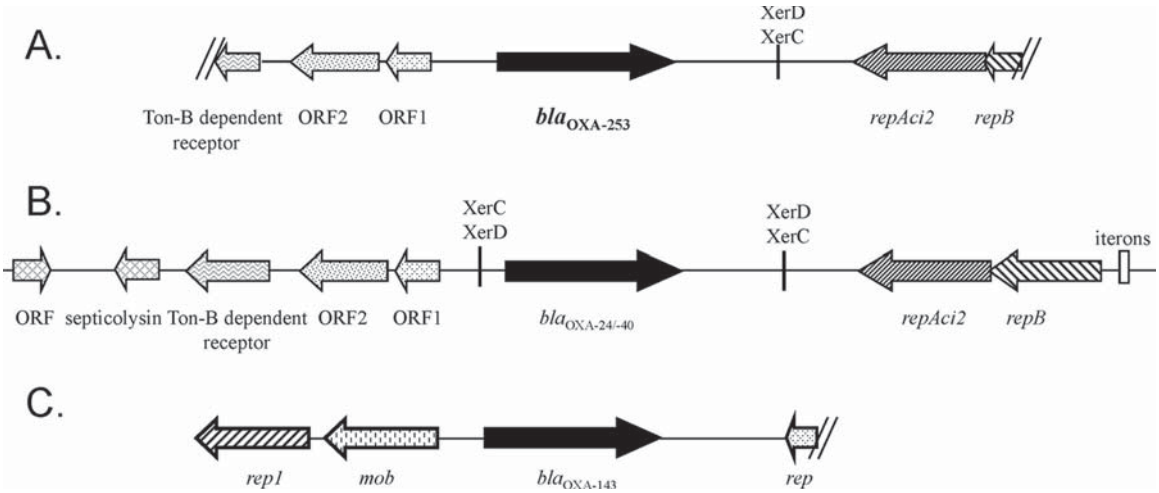

FIG 2 Schematic representation of the β-lactamase genes' genetic environment. (A) Genetic environment of the $bla_{OXA-253}$ gene in *A. baumannii* isolate 25; (B) previously identified structure surrounding the $bla_{OXA-24/-40}$ gene in pABVA01 (8); (C) previously identified structure surrounding the $bla_{OXA-143}$ gene in *A. baumannii* 135040 (2) (C).

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