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A novel OXA-10–like β-lactamase is present in different Enterobacteriaceae☆

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Abstract

OXA 101, a novel OXA-10 like enzyme, was found forming part of a class 1 integron located in a conjugative plasmid in three different species of *Enterobacteriaceae*. This β-lactamase is related to OXA-35 and OXA-56 and displays a narrow substrate hydrolysis profile.

Keywords: OXA-10 like; Gene cassette; Dissemination

OXA-10 and derivatives are class D β-lactamases that possess a variable substrate profile (Naas and Nordmann, 1999). Most of these oxacillinase genes are plasmid and/or integron located, while a few are associated to a chromosomal location (Flint and Schmitz, 1999; Naas and Nordmann, 1999). They have usually been described in gram-negative nonfermenting bacilli, mostly in *Pseudomonas aeruginosa* (Naas and Nordmann, 1999).

Citrobacter freundii (CF14), *Escherichia coli* (EC112), and *Enterobacter cloacae* (ECL153) were obtained from relevant urine samples at J.M. Cullen Hospital in Santa Fe, Argentina, in March 2005.

All were resistant to gentamicin, amikacin, ampicillin, amoxicillin-clavulanic acid, cephalotin, cefoxitin, cefotaxime, and trimethoprim–sulfamethoxazole. These isolates present high resistance levels to cefotaxime (512–1024 μg/mL), ceftazidime (128–256 μg/mL), gentamicin (>1024 μg/mL), and amikacin (>1024 μg/mL) (Clinical and Laboratory Standards Institute, 2009).

Crude extracts of CF14, EC112, and ECL153 rendered 2 β-lactam–hydrolyzing enzymes with apparent p*I* values of 5.4 and 7.2 after isoelectric focusing. In addition, CF14 and ECL153 presented another enzyme with p*I* of about 8 that could correspond to their intrinsic AmpC.

All isolates were positive (by PCR) for the presence of class 1 integrons with a single 1500-bp amplicon for variable region (VR).

Sequencing of the amplified VRs cloned in an appropriate vector (pGEM-T-Easy, Promega, Madison, WI) revealed the presence of the same 2 gene cassettes (accession number AM412777) forming part of the VRs of these class 1 integrons: in first place, $bla_{\text{oxa-101}}$, encoding a novel class D β-lactamase followed by the *aac(6′)-Ib* gene encoding an aminoglycoside acetyltransferase, which is capable of modificate aminoglycosides antibiotics.

When compared with other members of the OXA-10 family, OXA-101 had 2 nucleotide changes resulting in 2 amino acid substitutions (I89V and A230T) compared to OXA-56 (Poirel et al., 2004), and 4 nucleotide changes translated into 1 amino acid (S27F) substitution outside of the signal peptide region compared to OXA-35 (Aubert et al., 2001). $bla_{\text{OXA-35}}$ was also described forming part of the same gene cassette arrangement (followed by *aac(6′)-Ib*) in the VR of a class 1 integron (Aubert et al., 2001). In this cluster of OXA-10–derived restricted-spectrum

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Table 1
MICs of β-lactams for *Enterobacter cloacae* 153, transconjugant cells[a], and *Escherichia coli* HB101

β-Lactam	MIC (μg/mL)		
	Enterobacter cloacae 153	HB153[a]	*Escherichia coli* HB101
Ampicillin	>1024	64	1
Piperacillin	>256	8	0.5
Cephalotin	>1024	4	2
Cefoxitin	512	1	1
Ceftazidime	256	0.06	0.06
Cefotaxime	512	0.03	0.03
Cefepime	4	0.03	0.03
Imipenem	0.5	0.25	0.25

[a] Transconjugant cells. The 3 selected transconjugant cells had the same antibiotype.

β-lactamases, none of the postulated amino acid changes involved in the extension to their substrate profiles (N73S, G157D) (Mugnier et al., 1998) are present.

A mating assay was attempted in solid media using ECL153 as donor cell (F^+, AMP^R, STR^S) and *Escherichia coli* HB101 as the recipient one (F^-, AMP^s, STR^R). Transfer of plasmidic DNA was confirmed by PCR of *intI1* and $bla_{OXA-101}$ genes (OXA-101-B: GAAGGATCCATGAAAA-CATTTGCCGC, OXA-101-H: CTAACAAGCTTGCCAC-CAATGATGCC).

Plasmids of ECL153 and 3 selected transconjugants were extracted with a commercial kit (PhasePrep™ BAC DNA Kit, Sigma, St. Louis, MO).

Several plasmids of different sizes were detected in ECL153, whereas presence of only 1 high-molecular-weight plasmid was observed in transconjugants cells.

The presence of other β-lactamase (excluding bla_{OXA-10}) genes was evaluated by PCR. bla_{TEM} was detected in ECL153, but the corresponding amplicon was not obtained neither on the transconjugants nor on the recipient strain.

Antibiotic susceptibility of transconjugants is presented in Table 1. When comparing OXA-35 with OXA-101, susceptibility levels to different β-lactams were similar, even if these were analyzed in different genetic background in each case. For OXA-35, MICs values were determined for *Escherichia coli* XL1 harboring a recombinant high copy number plasmid that overexpressed OXA-35 (Aubert et al., 2001); instead, for OXA-101, MICs of β-lactams were determined for *Escherichia coli* HB101 transconjugants, which only had OXA-101 as β-lactam–hydrolyzing enzyme codified in a native low copy number plasmid. To the best of our knowledge, there are no available data for OXA-56 in a clean genetic background to compare.

The fact that we have found this novel narrow-spectrum OXA-β-lactamase in Enterobacteriaceae species can be explained after finding the same cassette arrangement in a class 1 integron located in a transferable plasmid.

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References

Aubert D, Poirel L, Ben Ali A, Goldstein F, Nordmann P (2001) OXA-35 is an OXA-10-related beta-lactamase from *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 48:717–721.

Clinical and Laboratory Standards Institute (CLSI) (2009) Performance standards for antimicrobial susceptibility testing; nineteenth informational supplement. Wayne, PA: CLSI.

Flint AC, Schmitz FJ (1999) Class 1 integrons, gene cassettes, mobility, and epidemiology. *Eur J Clin Microbiol Infect Dis* 18:761–770.

Mugnier P, Casin I, Bouthors A, Collatz E (1998) Novel OXA-10 derived extended spectrum beta-lactamases selected in vivo or in vitro. *Antimicrob Agents Chemother* 42:3113–3116.

Naas T, Nordmann P (1999) Oxa type beta-lactamases. *Curr Pharm Des* 5:865–879.

Poirel L, Magalhaes M, Lopes M, Nordmann P (2004) Molecular analysis of metallo-beta-lactamase gene bla_{SPM-1} surrounding sequences from disseminated *Pseudomonas aeruginosa* isolates in Recife, Brazil. *Antimicrob Agents Chemother* 48:1406–1409.