Intrapatient emergence of OXA-247: a novel carbapenemase found in a patient previously infected with OXA-163-producing *Klebsiella pneumoniae*

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Abstract

Two genetically related *Klebsiella pneumoniae* strains carrying OXA-type carbapenemases were isolated from a single patient 1 month apart. Kpn163 harboured OXA-163 and Kpn247 a new variant named OXA-247 that showed susceptibility to carbapenems and expanded-spectrum cephalosporins similar to OXA-48. Our epidemiological, biochemical and molecular results suggest the intrapatient emergence of $bla_{OXA-247}$ from $bla_{OXA-163}$.

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Carbapenem-hydrolyzing class D β-lactamases (CHDLs) hydrolyze carbapenems at low levels but do not have activity against extended-spectrum cephalosporins (ESCs) and aztreonam, which hinder their detection at the clinical microbiology laboratory [1,2]. Nowadays, CHDLs such as OXA-48-type enzymes are circulating among Mediterranean countries and are progressively disseminating to other geographical areas [3]. OXA-163 is an OXA-48-related enzyme with a weaker carpabenem activity but a higher hydrolytic activity against ESCs that has recently been reported in Argentina and later in Egypt (22nd European Congress of Clinical Microbiology and Infectious Diseases, Abstr. P1699) [4,5]. In Argentina, OXA-163 was first described in *Klebsiella pneumoniae* and *Enterobacter cloacae* [4]. Later, we described the broad spread of multiple clones of OXA-163-producing *K. pneumoniae* and *E. cloacae* from nine hospitals (22nd European Congress of Clinical Microbiology and Infectious Diseases, Abstr. P1699).

Here, we report the characterization of two *K. pneumoniae* clinical strains, isolated 1 month apart from the same patient, that belonged to the same clonal type but harboured different CHDLs: $bla_{OXA-163}$ and a new variant of this enzyme, $bla_{OXA-247}$.

A 15-year-old female diagnosed with acute lymphoid leukemia at the age of 3, was hospitalized in August 2010 and was subjected to a decolonization treatment with penicillin and colistin as part of a bone marrow transplant (BMT) protocol. Four days after BMT the patient developed febrile syndrome (FS) and sinusitis, and received empirical treatment with piperacillin-tazobactam, amikacin and vancomycin. Six days after, a positive blood culture was obtained with an expanded-spectrum-β-lactamase (ESBL)-producing *E. cloacae* resistant to amikacin; therefore the treatment was replaced by meropenem, colistin and amphotericin B. Twelve days later a new blood culture yielded a *K. pneumoniae* (Kpn163) suspicious for carbapenemase production. Susceptibility testing of Kpn163 (disk diffusion method and microdilution, Clinical and Laboratory Standards Institute-CLSI 2012 [6]), showed resistance to all β-lactams (including carbapenems), fosfomycin i.v. (European Committee on Antimicrobial Susceptibility Testing-EUCAST criteria) (http://www.eucast.org/clinical_breakpoints/), colistin, trimethoprim/sulphamethoxazole, chloramphenicol, tetracyclines and rifampin. Kpn163 remained susceptible to aminoglycosides, tigecycline (EUCAST) and quinolones. The treatment was replaced by gentamicin and ciprofloxacin with clinical success. A month later, a second BMT was performed and FS developed again. The patient was empirically treated with colistin and vancomycin but a week later, a second *K. pneumoniae* (Kpn247) was recovered from the paranasal sinuses. The susceptibility profile of Kpn247 was similar to that of Kpn163 with exceptions: the aminoglycosides and quinolones became resistant, and unexpectedly, Kpn247 restored the susceptibility to ESCs and aztreonam (Table 1). The patient died a day after Kpn247 isolation due to a massive infection with cytomegalovirus and graft vs. host disease. Both isolates were referred to

TABLE 1. Antimicrobial drug susceptibility, microbiological activity, isoelectric focusing and PCR of antimicrobial resistance determinants in the studied isolates, transcojugants and recipient strain

	Clinical isolates		Transconjugants		Acceptor
	K. pneumoniae Kpn163	*K. pneumoniae* Kpn247	*E. coli* TC163	*E. coli* TC247	*E. coli* J53
MICs in μg/ml					
Imipenem	8	16	0.5	0.5	0.06
Meropenem	32	16	0.03	0.03	0.03
Ertapenem	>256	>256	0.5	0.5	0.12
Cefotaxime	>256	1	128	0.12	0.12
Ceftazidime	>256	16	>256	2	0.12
Cefepime	>256	8	64	1	0.12
Aztreonam	128	32	64	8	0.12
Modified Hodge test to carbapenems					
MHT result	+	+	+	+	–
Isoelectric focusing					
pI bands	5.6; **6.7**; 7.6	5.6; **7**; 7.6	5.6; **6.7**	5.6; **7**	ND
Gene detected	PCR results				
$bla_{OXA-163}$	+	–	+	–	ND
$bla_{OXA-247}$	–	+	–	+	ND
bla_{TEM-1}	+	+	+	+	ND
bla_{SHV-1}	+	+	–	–	ND
aac(6′)Ib-cr	+	+	+	+	ND

MHT, modified Hodge test; ND, not determined; pI, isoelectric point; +, positive; –, negative.
pI bands of OXA-163 and OXA-247 are indicated in bold.

the National Reference Laboratory (INEI–Malbrán) for their characterization.

bla_{TEM}, bla_{SHV}, $bla_{OXA-163}$ and *aac(6′)-Ib-cr* were amplified by PCR in both Kpn163 and Kpn247 (Table 1). Locally prevalent β-lactamase (i.e. bla_{KPC}, bla_{PER}, bla_{CTX-M} and bla_{GES}) were PCR negative. *Pst*I-digested plasmidic DNA was ligated and amplified with outward primers to obtain the complete bla_{OXA} genes. Kpn163 harboured $bla_{OXA-163}$ and Kpn247 a new variant with two amino acid substitutions (Y219S and D220N, DBL numbering) assigned $bla_{OXA-247}$ (GenBank JX893517). The immediate genetic surroundings of both bla_{OXA} alleles were identical to that previously described [4] (i.e. bracketed upstream by the insertion sequence IS*4321* and downstream by a truncated IS*4*-like element; data not shown).

Both genes were transferred by biparental conjugation to *Escherichia coli* J53 (sodium azide-resistant) [7], rendering TC163 and TC247 from Kpn163 and Kpn247, respectively. Imipenem MICs of both transconjugants were eight times higher than that of the acceptor strain but the MICs of meropenem or ertapenem remained the same (Table 1). A modified Hodge test (MHT) to carbapenems [8] was positive for both transconjugants (Table 1).

Isoelectric focusing (IEF) [9] gave bands at pIs 5.6 (TEM) and 7.6 (SHV) in both strains, and pI 6.7 (OXA-163-Kpn163) and pI 7.0 (OXA-247-Kpn247) (Table 1). The shift of the pI from 6.7 to 7.0 when comparing OXA-163 and OXA-247 agrees with the substitution of the acidic aspartic acid residue to the neutral asparagine (D220N).

S1 nuclease digestion [10] showed that Kpn163 carried two plasmids of 70 Kb and 190 Kb while Kpn247 carried three plasmids of 70, 170 and 190 Kb in both isolates, the band of 70 Kb hybridized with the $bla_{OXA-163/247}$ probe. TC163 and TC247 carried a 70 Kb non-typeable plasmid (PCR-based replicon typing [11]) that hybridized with the $bla_{OXA-163/247}$ probe. These results suggest that both enzymes might be carried in the same plasmid.

Outer membrane protein-encoding genes, *ompK35* and *ompK36*, were amplified by PCR [12], sequenced and compared with *K. pneumoniae* MGH78578 [5]. *ompK*35 of both isolates were identical and showed five synonymous mutations and a 4-nucleotide insertion (CCAC) after codon 196, giving a deduced truncated product of 213 amino-acids. Kpn163 harboured a wild-type *ompK36* sequence while Kpn247 had a 5-nucleotide deletion at codons 44 and 45 that produced a premature termination codon with a deduced truncated product of 43 amino acids.

Pulsed-field gel electrophoresis (PFGE) studies revealed that Kpn163 and Kpn247 were clonally related, with only one band of difference in the macrorestriction pattern. Multilocus sequence typing (MLST) was performed according to the MLST Database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/) and both isolates belonged to ST37, also reported in a clinical isolate from Egypt expressing $bla_{OXA-163}$ [5].

In this case report we found the novel variant $bla_{OXA-247}$ in an immunosuppressed patient who received several antibiotic treatments and who was previously infected with a genetically related *K. pneumoniae* harbouring $bla_{OXA-163}$. Of note, to the best of our knowledge, the patient had not had any admissions to other health institutions from Buenos Aires City. In addition, this was the only report of OXA-type enzymes in

the hospital involved. This epidemiological setting in conjunction with the results of PFGE, plasmid content, genetic environment and porin analysis are consistent with the hypothesis of an intrapatient emergence of $bla_{OXA-247}$ from the previously established $bla_{OXA-163}$. The implications of OXA-247 in the local epidemiology remain to be determined.

Nucleotide Sequence Accession Number

The nucleotide sequence of $bla_{OXA-247}$ has been assigned GenBank accession number JX893517, (http://www.ncbi.nlm.nih.gov/genbank/index.html).

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Transparency Declaration

None to declare.

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