C2-084

Klebsiella pneumoniae Producing a New Variant Derived from Oxa-163: Case Report

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INTRODUCTION

Ambler class D β-lactamases include a large number of oxacillinases with only a few possessing carbapenemase activity (*JAC* 2012. 67:1597-1606). Most carbapenem hydrolyzing class D B-lactamases (CHDLs) hydrolyze carbapenems at low levels but do not have activity against broad-spectrum cephalosporins and aztreonam, which hinder their detection at the clinical microbiology laboratory level (AAC 2010. 54:24-38). Nowadays, CHDLs like OXA-48-type enzymes, are circulating among Mediterranean countries and are progressively disseminating to other geographical areas (Trends Mol Med. 2012. 18: 263-272). OXA-163 is an OXA-48-related-enzyme with a weaker carbapenem activity but a higher hydrolytic activity against cephalosporins (AAC 2011. 55: 2546-2551) that has recently been reported in Argentina and later in Egypt (JCM. 2012. 50: 2489-2491, F. Pasteran, *ECCMID* 2012). In Argentina OXA-163 was first described in *Klebsiella pneumoniae* and *Enterobacter cloacae* (AAC. 2012. 55: 2546-2551) and latter in multiple clones of these species (Pasteran, *ECCMID* 2012).

OBJECTIVES

To report the case of a patient infected in two episodes with *K. penumoniae* producing two OXA-type carbapenemases

RESULTS

FIGURE 2.- Amino acid sequence of $bla_{OXA-247}$

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>bla_OXA247, 261 aa
MRVLALSAVFLVASIIGMPAVAKEWQENKSWNAHFTEHKSQGVVVLWNENKQQGFTNNLK   60

RANQAFLPASTFKIPNSLIALDLGVVKDEHQVFKWDGQTRDIATWNRDHNLITAMKYSVV  120
                70.                                        DBL
PVYQEFARQIGEARMSKMLHAFDYGNEDISGNVDSFWLDGGIRISATEQISFLRKLYHNK  180

LHVSERSQRIVKQAMLTEANGDYIIRAKTGSNTKIGWWVGWVELDDNVWFFAMNMDMPTS  240
                      217.                                  DBL
                        219.
                          220.
DGLGLRQAITKEVLKQEKIIP                                        261

Y219S, D220N
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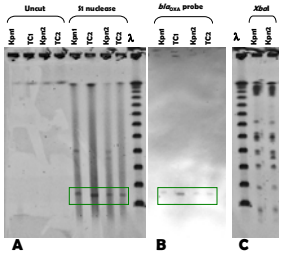
Kpn2 carries a new variant derived from $bla_{OXA-163}$ with two amino acid substitutions (Y219S, D220N – DBL numbering) assigned $bla_{OXA-247}$ (Kpn2-$bla_{OXA-247}$).

FIGURE 2. Molecular characterization of Kpn1-$bla_{OXA-163}$ and Kpn2-$bla_{OXA-247}$



(A) S1 nuclease digestion of Kpn1-$bla_{OXA-163}$; Kpn1 carried two plasmids of approximately 70 Kb and 192 Kb. Kpn2-$bla_{OXA-247}$, carried three plasmids of: 70, 172 and 191 Kb approximately.
(B) Specific $bla_{OXA-163/247}$ probe hybridized with the band of 70Kb of both clinical and TC strains.
(C) PFGE of Kpn1 and Kpn2 revealed that they were clonally related (one band of difference) in the *Xba*I macrorestriction pattern.

Table 1. Antimicrobial susceptibility (MICs), microbiological activity against carbapenems and PCR of antimicrobial resistance determinants in the studied isolates, transcojugants and recipient strain.

	Clinical isolates				Transconjugants		Acceptor
	K. pneumoniae		*K. pneumoniae*		*E. coli*	*E. coli*	*E. coli*
Strain number	**Kpn1-$bla_{OXA-163}$**		**Kpn2-$bla_{OXA-247}$**		**TC1**	**TC2**	**J53**
Antibiotics	**MICs (µg/ml)**						
Imipenem	8	R	16	R	0.5	0.5	0.06
Meropenem	32	R	16	R	0.03	0.03	0.03
Ertapenem	>256	R	>256	R	0.5	0.5	0.12
Cefotaxime	>256	R	1	S	128	0.12	0.12
Ceftazidime	>256	R	16	R	>256	2	0.12
Cefepime	>256	R	8	S	64	1	0.12
Aztreonam	128	R	32	R	64	8	0.12
	Modified Hodge Test to Imipenem						
MHT result	+		+		+	+	-
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

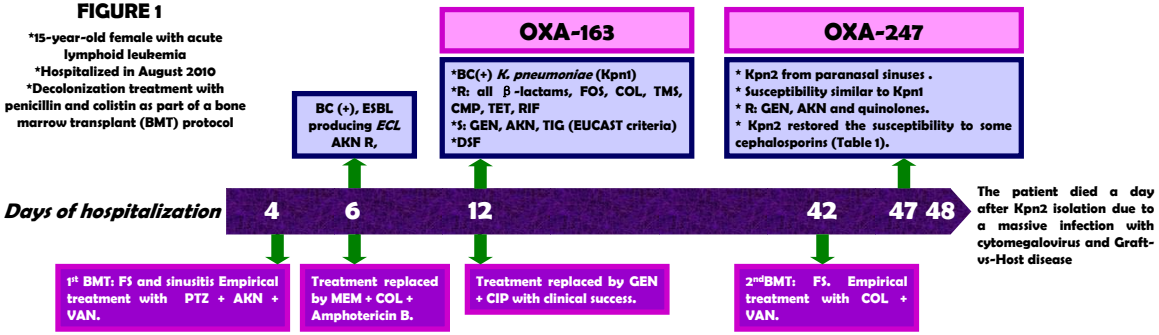
ND not determined, + positive, - negative

TC1: Transconjugant from Kpn1-$bla_{OXA-163}$, TC2 from Kpn2-$bla_{OXA-247}$

CASE REPORT

FIGURE 1

*15-year-old female with acute lymphoid leukemia
*Hospitalized in August 2010
*Decolonization treatment with penicillin and colistin as part of a bone marrow transplant (BMT) protocol



The patient died a day after Kpn2 isolation due to a massive infection with cytomegalovirus and Graft-vs-Host disease

BC, blood culture; BMT, bone marrow transplant, FS, febrile syndrome, ECL, *Enterobacter cloacae*; PTZ, piperacillin-tazobactam; AKN, amikacin; VAN, vancomycin; MEM, meropenem, COL, colistin; GEN, gentamicin; CIP, ciprofloxacin, FOS, fosfomycin; TMS, trimethoprim/sulfamethoxazole; CMP, chloramphenicol; TET, tetracycline; RIF, rifampin TIG, tigecycline. DSF: decreased susceptibility to fluorquinolones.

MATERIALS AND METHODS

Clinical isolates and antimicrobial susceptibility testing. Susceptibility testing was performed by the disk diffusion method and microdilution, (CLSI criteria). *Molecular assays.* Presence of ESBLs, PMQR and acetylase genes were tested by specific PCRs using standard conditions. The complete bla_{OXA} gene was obtained by the reverse PCR method (FIGURE 2), briefly: HindIII restriction fragments obtained from purified plasmids were ligated and amplified by PCR with outward primers (OXA-163-Rout- CTCATTCCAGAGCACAACTACGCCCTGTGA;OXA-163-Fout-CGTATCGGAGCGCAGCCAGCGTATTGTCA). DNA sequencing was performed using the BigDye terminator methodology. The localization of $bla_{OXA-163}$ and $bla_{OXA-247}$ (FIGURE 3) was investigated using S1 nuclease digestion, followed by PFGE and posterior hybridization with a $bla_{OXA-163/247}$ probe (Anal Biochem. 1995. 226: 235-240). *Biparental conjugation.* Agar mating method was used to transfer resistance determinants from both isolates to sodium azide-resistant *E. coli* J53 recipient. Ampicillin (AMP, 50 µg/ml) plus sodium azide (200 µg/ml) were used to select for transconjugants. Pulse Field Gel Electrophoresis (PFGE): total DNA was digested with XbaI. PFGE conditions and analysis criteria was done as explained elsewhere J Clin Microbiol. 1995. 33: 2233-2239. Clin Microbiol Infect. 2011. 17: 1520-1524

CONCLUSIONS

1.- This work describes the intra-treatment emergence of a novel CHDL OXA-247 in a patient previously carrying OXA-163 (Figure 1 and 2).
2.- $bla_{OXA-247}$ showed similar carbapenem hydrolysis than $bla_{OXA-163}$ but lower activity against cephalosporins (Table 1), making the hydrolytic properties of $bla_{OXA-247}$ more similar to bla_{OXA-48}.
3.-Both enzymes might be carried on the same plasmid (Figure 2 and 3). Further studies are necessary to understand the mobilization process that leads to the dissemination of these genes.
4. The implications of OXA-247 in the local epidemiology remain to be determined.