

Characterization of a New blaOXA-48-Carrying Plasmid in Enterobacteriaceae

Sibel Berger, Corentine Alauzet, Nejla Aissa, Sandrine Henard, Christian Rabaud, Richard Bonnet, Alain Lozniewski

► **To cite this version:**

Sibel Berger, Corentine Alauzet, Nejla Aissa, Sandrine Henard, Christian Rabaud, et al.. Characterization of a New blaOXA-48-Carrying Plasmid in Enterobacteriaceae. Antimicrobial Agents and Chemotherapy, American Society for Microbiology, 2013, 57 (8), pp.4064 - 4067. <<http://aac.asm.org/content/57/8/4064>>. <10.1128/AAC.02550-12>. <hal-01480994>

HAL Id: hal-01480994

<https://hal.univ-lorraine.fr/hal-01480994>

Submitted on 2 Mar 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Characterization of a New *bla*_{OXA-48}-Carrying Plasmid in *Enterobacteriaceae*

Sibel Berger,^a Corentine Alauzet,^a Nejla Aissa,^a Sandrine Hénard,^b Christian Rabaud,^b Richard Bonnet,^c Alain Lozniewski^a

Laboratoire de Bactériologie, CHU de Nancy, Nancy, France^a; Service des Maladies Infectieuses et Tropicales, CHU de Nancy, Nancy, France^b; Laboratoire de Bactériologie, CHU de Clermont-Ferrand, Clermont-Ferrand, France^c

In this work, we characterized a new, 160-kb, *bla*_{OXA-48}-harboring IncL/M-type plasmid isolated from a *Klebsiella pneumoniae* strain from France. Moreover, we report the transfer of a 60-kb OXA-48-encoding plasmid from *Klebsiella pneumoniae* to other *Enterobacteriaceae* in two patients.

The emergence of carbapenemase-producing *Enterobacteriaceae* has become a major public health concern (1). Carbapenemases identified in *Enterobacteriaceae* are frequently class A β-lactamases, particularly KPC enzymes, and class B metallo-β-lactamases. However, the emergence of *Enterobacteriaceae* strains producing class D carbapenemases such as OXA-48 has increasingly been reported (2). This enzyme, initially detected in a carbapenem-resistant *Klebsiella pneumoniae* strain in Turkey (3), has been identified extensively in that country as well as in other surrounding Mediterranean countries (2, 4). Since then, it has also been identified in Senegal, Kuwait, the Sultanate of Oman, and India, as well as in several European countries (2, 5–10). Although OXA-48 has most often been found in *K. pneumoniae*, it has also been identified in *Escherichia coli*, *Enterobacter* spp., *Klebsiella oxytoca*, *Citrobacter freundii*, *Providencia rettgeri*, and *Serratia marcescens* (2, 7, 8, 10, 11). In these strains, *bla*_{OXA-48} is usually associated with a Tn1999-type transposon (12) and has been observed in self-conjugative 60- to 70-kb plasmids exhibiting similar restriction profiles (5, 6, 11, 12). It is considered that the current spread of *bla*_{OXA-48} is linked to the diffusion of a unique self-conjugative IncL/M plasmid, designated pOXA-48a, that was recently sequenced by Poirel et al. (13). However, the horizontal spread of *bla*_{OXA-48}-harboring plasmids in a single patient has not yet been reported.

In this work, we report the transfer of a 60-kb OXA-48-encoding plasmid from *K. pneumoniae* to other *Enterobacteriaceae* in two patients. Moreover, we characterized a new, 160-kb, *bla*_{OXA-48}-carrying conjugative plasmid from a *K. pneumoniae* isolate.

In 2010, we isolated six *Enterobacteriaceae* organisms resistant to ertapenem (MICs, 1.5 to 3 μg/ml) (Table 1) from samples from two patients hospitalized at the University Hospital Center of Nancy, France. There was no direct epidemiological link between the two patients, who were both colonized during an outbreak which affected our hospital center between 2010 and 2012 (10). For patient 1 (a 61-year-old man with type 2 diabetes), ertapenem-resistant *K. pneumoniae* KP1 was isolated from an intra-abdominal abscess and feces in October 2010. Two additional ertapenem-resistant strains, designated *K. pneumoniae* KP1a and *E. coli* EC1, were recovered 2 months later, from rectal and perineal wound swabs, respectively. For patient 2 (a 64-year-old man with type 2 diabetes who was admitted for acute cholangitis), the ertapenem-resistant *K. pneumoniae* strain KP2 was isolated from blood cultures, abdominal samples, and rectal swabs. Two months

later, rectal swabs evidenced fecal carriage of two additional ertapenem-resistant *Enterobacteriaceae*: *Enterobacter cloacae* ECL2 and *K. oxytoca* KO2. MICs of β-lactams were determined by Etest (bioMérieux, Marcy l'Etoile, France) and interpreted according to CLSI guidelines (14). All strains displayed MIC values within the susceptible range for meropenem and doripenem (Table 1). A decreased susceptibility to imipenem was observed for EC1 and KO2. The six strains were resistant to penicillins but were susceptible to cefotaxime and ceftazidime, except for KP1, KP2, and ECL2. Extended-spectrum β-lactamase (ESBL) phenotypic detection was based on the use of combined double-disk synergy tests on Mueller-Hinton agar with and without cloxacillin, an AmpC inhibitor (15). The synergy test evidenced the production of an ESBL in KP1 and KP2, while ECL2 exhibited a phenotypic profile indicative of a high level of cephalosporinase expression. The modified Hodge test was positive for all strains.

Molecular detection of β-lactamase-encoding genes was performed by using the Check-Points MDR CT102 array system (Check-Points, Wageningen, The Netherlands) (16). This DNA microarray hybridization suggested the presence of *bla*_{OXA-48} in all strains, as well as its association with *bla*_{CTX-M-1-group} in strains KP1 and KP2. The results were confirmed by specific PCR experiments and sequencing, using primers oxa-48F (5'-AAGGAATG GCAAGAAAAACAAAA-3') and oxa-48R (5'-CCATAATCGAAA GCATGTAGCA-3') for *bla*_{OXA-48} and primers ctx-mF (5'-ATCT GTTAAATCAGCGAGTTGAGAT-3') and ctx-mR (5'-GTATTG CCTTTCATCCATGTCAC-3') for *bla*_{CTX-M-1-group}.

The strains were compared by pulsed-field gel electrophoresis (PFGE) with XbaI-digested genomic DNAs and a CHEF-DR III instrument (Bio-Rad, Marnes-la-Coquette, France). According to the criteria of Tenover et al. (17), KP1 and KP2 were clonally related, while KP1a displayed a different pulsotype. EC1 (from patient 1), which was resistant to ertapenem and intermediate to imipenem, exhibited the same PFGE profile as an *E. coli* isolate

Received 17 December 2012 Returned for modification 15 February 2013

Accepted 28 May 2013

Published ahead of print 3 June 2013

Address correspondence to Alain Lozniewski, a.lozniewski@chu-nancy.fr.

Copyright © 2013, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AAC.02550-12

TABLE 1 Phenotypic and genotypic characteristics of OXA-48-producing isolates obtained from patients 1 and 2 and of transformants, transconjugants, and corresponding recipient strains used in this study

Strain	Presence of <i>bla</i> _{OXA-48} -carrying plasmid		MIC (μg/ml)				Modified Hodge test result
	60 kb	160 kb	Imipenem	Ertapenem	Meropenem	Doripenem	
Clinical isolates							
<i>K. pneumoniae</i> KP ^a	+	–	0.75	3	0.38	0.5	+
<i>K. pneumoniae</i> KP1a	–	+	0.5	2	0.38	0.38	+
<i>E. coli</i> EC1	+	–	2	3	0.38	0.75	+
<i>E. coli</i> EC1a	–	–	0.19	0.012	0.012	0.016	–
<i>K. pneumoniae</i> KP2 ^a	+	–	0.75	4	0.75	0.75	+
<i>E. cloacae</i> ECL2	+	–	6	3	0.38	0.25	+
<i>K. oxytoca</i> KO2	+	–	0.75	1.5	0.75	0.25	+
Other strains							
<i>E. coli</i> DH10B	–	–	0.19	0.012	0.016	0.023	–
<i>E. coli</i> DH10B-T1	+	–	0.75	4	0.38	0.38	+
<i>E. coli</i> DH10B-T2	–	+	0.75	2	0.38	0.25	+
<i>E. cloacae</i> LBN600	–	–	0.25	0.25	0.023	0.032	–
<i>E. cloacae</i> LBN600-T1	–	+	0.25	3	0.25	0.25	+
<i>K. oxytoca</i> LBN548	–	–	0.19	0.023	0.016	0.023	–
<i>K. oxytoca</i> LBN548-T1	–	+	0.38	4	0.38	0.38	+

^a Isolate harboring a *bla*_{CTX-M-1-group}-carrying plasmid.

(EC1a) which was obtained from the perineal wound of patient 1 and was susceptible to all carbapenems tested.

The plasmid contents of strains were visualized after DNA linearization by the S1 nuclease followed by PFGE migration as described previously (18). S1-PFGE digests were transferred to a nylon membrane and hybridized with PCR-based probes labeled by use of a PCR DIG probe synthesis kit (Roche Diagnostics, Meylan, France). KP1 and KP2 contained a 50-kb plasmid carrying *bla*_{CTX-M-1-group}. The hybridization specific to *bla*_{OXA-48} revealed the presence of a 60-kb plasmid carrying *bla*_{OXA-48} (pKPoxa-48N1) in both strains KP1 and EC1 of patient 1 and all strains of patient 2 (KP2, ECL2, and KO2) (Table 1). Although OXA-48 production has been observed mainly in *K. pneumoniae*, recent reports have shown that this gene may be encountered in other enterobacterial species. It has been hypothesized that this dissemination is probably related to the fact that *bla*_{OXA-48} is located on a transferable plasmid. The sequence of clinical events observed in this work and the microbiological findings strongly support the hypothesis that this interspecies spread occurred as the result of the mobilization of a 60-kb plasmid carrying *bla*_{OXA-48} in both patients.

However, Southern hybridization with strain KP1a indicated that *bla*_{OXA-48} was present, surprisingly, on a large plasmid of 160 kb (pKPoxa-48N2). This new OXA-48-encoding plasmid transferred into a cefoxitin-resistant *E. cloacae* strain (LBN600) and a sulfamethoxazole-trimethoprim-resistant *K. oxytoca* strain (LBN548) by liquid conjugation in Luria-Bertani (LB) broth at a donor/recipient ratio of 1:5 for 3 h at 37°C. The resulting transconjugants exhibited a significant increase of ertapenem MIC values (Table 1), and the presence of pKPoxa-48N2 was confirmed by S1-PFGE and *bla*_{OXA-48} hybridization. This result shows that the spread of *bla*_{OXA-48} in *Enterobacteriaceae* can be mediated by an additional plasmid.

The two transformants DH10B-T1 and DH10B-T2, containing pKPoxa-48N1 (60 kb) and pKPoxa-48N2 (160 kb), respec-

tively, were obtained on LB agar plates containing 4 μg/ml ertapenem by the electroporation of KP1 and KP1a plasmid contents into *E. coli* DH10B by use of a MicroPulser electroporator (Bio-Rad). The complete nucleotide sequences of these plasmids were determined using a Roche Life Sciences 454 FLX genome sequencer platform (GATC Biotech, Konstanz, Germany). Sequencing generated 17,000 sequences (mean sequence size, 400 bp) for pKPoxa-48N1 and 19,000 sequences (mean sequence size, 390 bp) for pKPoxa-48N2. Sequences were assembled into one contig for pKPoxa-48N1 and into seven contigs for pKPoxa-48N2. The remaining gaps were closed by sequencing the PCR products obtained with primers designed from the extremities of each contig.

The complete sequencing of pKPoxa-48N1 showed that this circular plasmid of 62,583 bp (mean GC content of 51.1%) was an IncL/M plasmid which exhibited features very similar to those of pCTX-M360 (accession number NC_011641) and pOXA-48a from *K. pneumoniae* (accession number JN626286) at both the sequence and gene organization levels. The *bla*_{OXA-48} gene was the only antibiotic resistance gene present in pKPoxa-48N1. The overall sequence similarities between the common parts of this plasmid and pCTX-M360 or pOXA-48a were higher than 98% or 99%, respectively. The significant differences between pKPoxa-48N1 and pCTX-M360 were the absence of two transposable elements, i.e., a Tn2-type transposon containing *bla*_{TEM-1} and ISEcp1, associated with the *bla*_{CTX-M-3} gene (19), and the replacement of ISEcp1-*bla*_{CTX-M-3} by an IS1999-based composite transposon harboring *bla*_{OXA-48}. In contrast to pOXA-48a, pKPoxa-48N1 contained an ISIR element inserted into the IS1999 element located upstream of *bla*_{OXA-48}, as observed in the composite transposon Tn1999.2 (11).

Total sequencing of pKPoxa-48N2 revealed an IncL/M plasmid of 167,194 bp with a mean GC content of 51%. The region containing *bla*_{OXA-48} (around 62.6 kb) was identical to that in pKPoxa-48N1 (Fig. 1). No other antibiotic resistance gene was

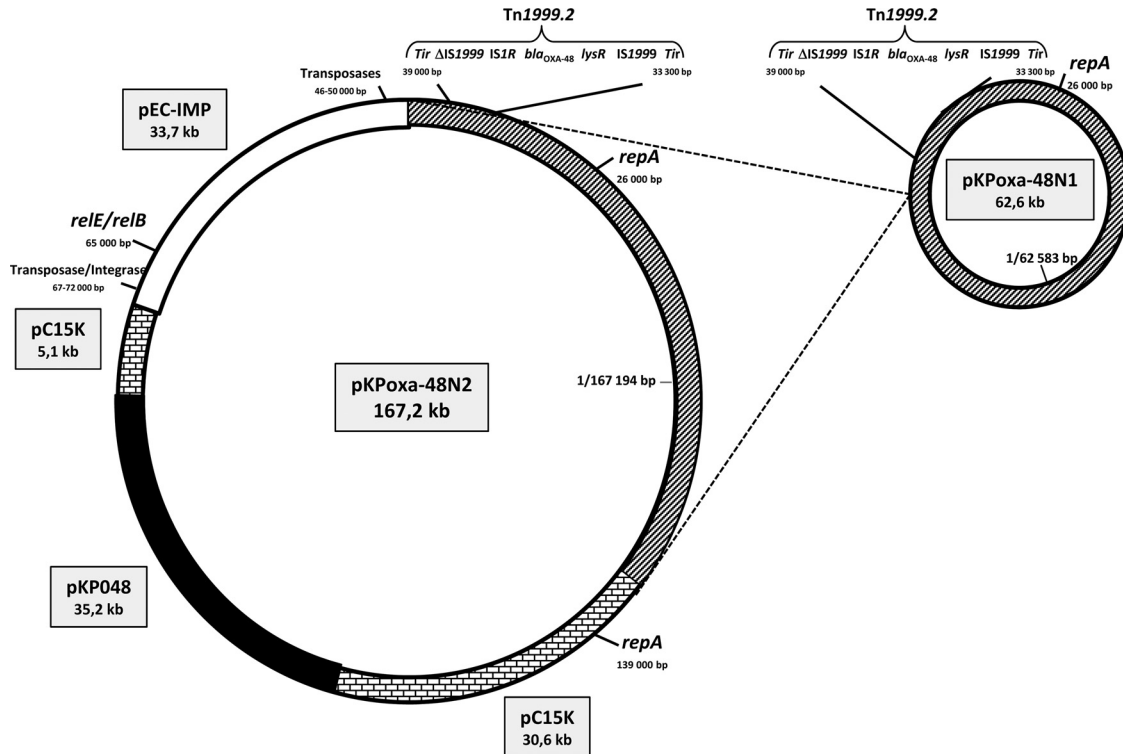


FIG 1 Schematic representation of the modular organization of pKPoxa-48N2 according to regions which are homologous to fragments from different plasmids: pKPoxa-48N1 (harboring *bla*_{OXA-48}), pEC-IMP (harboring *relE* and *relB*), pC15K, and pKP048. Molecular sizes of the homologous fragments are indicated in kb.

found within pKPoxa-48N2. BLAST analysis showed that this plasmid consisted of three other regions, of 33.7, 35.7, and 35.2 kb, exhibiting high similarities (>98%) with respective regions of similar size in the plasmid pEC-IMP (318 kb; accession number NC_012555), found in *E. cloacae*, and two plasmids, pc15-k (96 kb; accession number NC_015154) and pKP048 (151 kb; accession number NC_014312), found in *K. pneumoniae*. The 62.2-kb region containing *bla*_{OXA-48} and the 33.7-kb pEC-IMP-like region were inserted into the 35.7-kb pc15-k-like region. pKPoxa-48N2 therefore resulted merely from multiple rearrangements involving different plasmids originating from different enterobacterial species. As for pKPoxa-48N1, *bla*_{OXA-48} was the only resistance gene observed in pKPoxa-48N2. Thus, all other resistance genes usually harbored by pEC-IMP, pc15-k, and pKP048 were lost during the events that led to the selection of pKPoxa-48N2. Interestingly, we observed that in contrast to pKPoxa-48N1, this plasmid harbored genes previously reported to encode the toxin-antitoxin systems RelE-StbE and RelB-DinJ (20). These genes, which were found to be located within the 33.7-kb pEC-IMP-like region, may therefore be responsible for a higher stability of pKPoxa-48N2 in bacteria.

In conclusion, we observed for the first time that a 60-kb *bla*_{OXA-48}-harboring plasmid can be mobilized in different strains of *Enterobacteriaceae* species *in vivo*. We also showed that the mobilization of this gene among *Enterobacteriaceae* is not restricted to a single plasmid and may also rely on a 160-kb plasmid.

Nucleotide sequence accession numbers. Complete nucleotide sequences of pKPoxa-48N1 and pKPoxa-48N2 were depos-

ited in the GenBank database under accession numbers [KC757416](https://www.ncbi.nlm.nih.gov/nuccore/KC757416) and [KC757417](https://www.ncbi.nlm.nih.gov/nuccore/KC757417), respectively.

ACKNOWLEDGMENT

We are grateful to Isabelle Scholtus for her technical help.

REFERENCES

- Nordmann P, Dortet L, Poirel L. 2012. Carbapenem resistance in *Enterobacteriaceae*: here is the storm! *Trends Mol. Med.* 18:263–272.
- Poirel L, Potron A, Nordmann PP. 2012. OXA-48-like carbapenemases: the phantom menace. *J. Antimicrob. Chemother.* 67:1597–1606.
- Poirel L, Héritier C, Tolün V, Nordmann P. 2004. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* 48:15–22.
- Hammerum AM, Larsen AR, Hansen F, Justesen US, Friis-Møller AE, Lemming LE, Fuursted K, Littauer P, Schønning K, Gahrn-Hansen B, Ellermann-Eriksen S, Kristensen B. 2012. Patients transferred from Libya to Denmark carried OXA-48-producing *Klebsiella pneumoniae*, NDM-1-producing *Acinetobacter baumannii* and methicillin-resistant *Staphylococcus aureus*. *Int. J. Antimicrob. Agents* 40:191–192.
- Cuzon G, Ouanich J, Gondret R, Naas T, Nordmann P. 2011. Outbreak of OXA-48-positive carbapenem-resistant *Klebsiella pneumoniae* isolates in France. *Antimicrob. Agents Chemother.* 55:2420–2423.
- Cuzon G, Naas T, Bogaerts R, Glupczynski Y, Huang TD, Nordmann P. 2008. Plasmid-encoded carbapenem-hydrolyzing β -lactamase OXA-48 in an imipenem-susceptible *Klebsiella pneumoniae* strain from Belgium. *Antimicrob. Agents Chemother.* 52:3463–3464.
- Dimou V, Dhanji H, Pike R, Livermore DM, Woodford N. 2012. Characterization of *Enterobacteriaceae* producing OXA-48-like carbapenemases in the UK. *J. Antimicrob. Chemother.* 67:1660–1665.
- Dortet L, Poirel L, Al Yaquobi F, Nordmann P. 2012. NDM-1, OXA-48 and OXA-181 carbapenemase-producing *Enterobacteriaceae* in Sultanate of Oman. *Clin. Microbiol. Infect.* 18:E144–E148.

9. Poirel L, Carbonnelle E, Bernabeu S, Gutmann L, Rotimi V, Nordmann P. 2012. Importation of OXA-48-producing *Klebsiella pneumoniae* from Kuwait. *J. Antimicrob. Chemother.* 67:2051–2052.
10. Vaux S, Carbone A, Thiolet JM, Jarlier V, Coignard B, RAISIN and Expert Laboratories Groups. 2011. Emergence of carbapenemase-producing *Enterobacteriaceae* in France, 2004 to 2011. *Euro Surveill.* 16:19880. <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19880>.
11. Carrër A, Poirel L, Yilmaz M, Akan OA, Feriha C, Cuzon G, Matar G, Honderlick P, Nordmann P. 2010. Spread of OXA-48-encoding plasmid in Turkey and beyond. *Antimicrob. Agents Chemother.* 54:1369–1373.
12. Carrër A, Poirel L, Eraksoy H, Cagatay AA, Badur S, Nordmann P. 2008. Spread of OXA-48-positive carbapenem-resistant *Klebsiella pneumoniae* isolates in Istanbul, Turkey. *Antimicrob. Agents Chemother.* 52:2950–2954.
13. Poirel L, Bonnin RA, Nordmann P. 2012. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. *Antimicrob. Agents Chemother.* 56:559–562.
14. Clinical and Laboratory Standards Institute. 2011. Performance standards for antimicrobial susceptibility testing. CLSI M100-S21. Clinical and Laboratory Standards Institute, Wayne, PA.
15. European Committee on Antimicrobial Susceptibility Testing. 2012. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Consultation/EUCAST_guidelines_detection_of_resistance_mechanisms_121222.
16. Naas T, Cuzon G, Bogaerts P, Glupczynski Y, Nordmann P. 2011. Evaluation of a DNA microarray (Check-MDR CT102) for rapid detection of TEM, SHV, and CTX-M extended-spectrum β -lactamases and of KPC, OXA-48, VIM, IMP, and NDM-1 carbapenemases. *J. Clin. Microbiol.* 49:1608–1613.
17. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial typing. *J. Clin. Microbiol.* 33:2233–2239.
18. Barton BM, Harding GP, Zuccarelli AJ. 1995. A general method for detecting and sizing large plasmids. *Anal. Biochem.* 226:235–240.
19. Zhu WH, Luo L, Wang JY, Zhuang XH, Zhong L, Liao K, Zeng Y, Lu YJ. 2009. Complete nucleotide sequence of pCTX-M360, an intermediate plasmid between pEL60 and pCTX-M3, from a multidrug-resistant *Klebsiella pneumoniae* strain isolated in China. *Antimicrob. Agents Chemother.* 53:2591–2593.
20. Chen YT, Liao TL, Liu YM, Lauderdale TL, Yan JJ, Tsai SF. 2009. Mobilization of *qnrB2* and *ISCR1* in plasmids. *Antimicrob. Agents Chemother.* 53:1235–1237.