AAC
Journals.ASM.org

Genetic Features of the Widespread Plasmid Coding for the Carbapenemase OXA-48

Laurent Poirel, Rémy A. Bonnin, and Patrice Nordmann

Service de Bactériologie-Virologie, INSERM U914, Emerging Resistance to Antibiotics, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine et Université Paris-Sud, K.-Bicêtre, France

Complete sequencing of plasmid pOXA-48a carrying the bla_{OXA-48} gene from a *Klebsiella pneumoniae* isolate was performed. Its backbone corresponded to that of an IncL/M-type plasmid, in which the bla_{OXA-48} gene had been integrated through the acquisition of the Tn*1999* composite transposon without any other antibiotic resistance gene. Molecular epidemiology using a collection of international OXA-48 producers revealed the wide diffusion of pOXA-48a or closely related plasmids.

Carbapenem-hydrolyzing β-lactamases belonging to Ambler classes A, B, and D have been found worldwide among the *Enterobacteriaceae* (24). Although carbapenem-hydrolyzing class D β-lactamases (CHDLs) are identified mainly in *Acinetobacter* species (19, 23), OXA-48 has been identified only in the *Enterobacteriaceae*. The bla_{OXA-48} gene was first identified in a *Klebsiella pneumoniae* isolate from Turkey (22). Since then, several other OXA-48-producing isolates of various enterobacterial species (including *Citrobacter freundii* and *Escherichia coli*) have been reported, first mainly in Turkey and then in Belgium, France, Lebanon (16), Egypt, Israel (12), Senegal (18), Morocco (25), and Tunisia (9). In addition, there have been recent reports of OXA-48-related enzymes, such as OXA-181, being identified in India (7) and in other countries but always with a link to India (26), as well as OXA-163 from Argentina (21). It is noteworthy that OXA-48 and OXA-181 hydrolyze penicillins and carbapenems but spare extended-spectrum cephalosporins (27, 28). Many OXA-48 producers coexpress an extended-spectrum β-lactamase (ESBL) (most often CTX-M-15 or SHV-12), making those isolates resistant to all β-lactams available.

Previous studies indicated that plasmids carrying the bla_{OXA-48} gene from different enterobacterial isolates, different clones, and different countries may share very similar features (6, 10). They were self-conjugative, very similar in size (reported to be ca. 60 to 70 kb), did not encode additional resistance markers, and were not typeable by using PCR-based replicon typing (PBRT) (4). This suggested the possibility of a wide spread of a single plasmid at the origin of dissemination of this carbapenemase gene. This prompted us to determine the sequence of one of these plasmids, to compare the identified structure with those of other OXA-48-positive plasmids, and to determine if some particular features could explain its successful spread. It is noteworthy that a 7.6-kb plasmid carrying the $bla_{OXA-181}$ gene and recovered from a *K. pneumoniae* isolate from Oman was fully sequenced. This revealed that it belonged to the broad-host-range ColE family of plasmids and that the $bla_{OXA-181}$ gene had been acquired by an IS*Ecp1*-related one-ended transposition mechanism (27), two features clearly differentiating it from the bla_{OXA-48} plasmids.

Plasmid pOXA-48a was extracted from the *E. coli* transconjugant recovered from the *K. pneumoniae* 11978 clinical isolate (22) by using the Qiagen Maxi kit (Qiagen, Courtaboeuf, France). The complete sequencing work flow of the Illumina genome analyzer IIx system was performed by the DNA Vision company (Gosselles, Belgium).

Sequence analysis of pOXA-48a revealed that it was 61,881 bp in size, with an average G+C content of 51.1% (Fig. 1). Each predicted protein was compared against the GenBank protein database using BlastP (http://blast.ncbi.nlm.nih.gov/Blast.cgi) with a minimum cutoff of 30% identity over 80% length coverage. Gene sequences were further compared and aligned with GenBank data using BlastN and ClustalW software. The IncL/M plasmids pCTX-M3 (GenBank accession number AF550415), which harbors the $bla_{CTX-M-3}$ ESBL gene, and pHK-NDM (GenBank accession number HQ451074), which harbors the metallo-β-lactamase bla_{NDM-1} gene, were used as references for annotating pOXA-48a.

In silico analysis showed that pOXA-48a possessed the highest degree of sequence identity and gene synteny within its entire scaffold with the reference plasmid pCTX-M3 (97% identity at the nucleotide level) (11), with the exception of two loci. One region that differs from pCTX-M3 corresponded to Tn*1999*, which consists of two copies of the same insertion sequence, IS*1999*, flanking a DNA fragment that includes the bla_{OXA-48} gene (1, 21). As previously shown, transposon Tn*1999* was inserted within a *tir* gene similar to that responsible for inhibition of transfer of plasmid R100 (17). A target site duplication of 9 bp (CGTTCAGCA) was identified on each extremity of Tn*1999* (1) (Fig. 2). Downstream of bla_{OXA-48}, a *lysR* gene encoding a regulatory protein of 304 amino acids was identified (98% amino acid identity with that of *Shewanella oneidensis* MR1) (13), followed by a truncated fragment of a gene encoding an acetyl coenzyme A carboxylase sharing 100% amino acid identity with that of *S. oneidensis* MR1 (13). In pCTX-M3, the IS*Ecp1*-$bla_{CTX-M-3}$ transposable locus was inserted close to this target site for Tn*1999*, suggesting that this locus could possibly be a hot spot for integrating foreign DNA within IncL/

Received 13 July 2011 **Returned for modification** 15 August 2011
Accepted 2 November 2011
Published ahead of print 14 November 2011
Address correspondence to Patrice Nordmann, nordmann.patrice@bct.aphp.fr.
L. Poirel and R. A. Bonnin contributed equally to this work.

doi:10.1128/AAC.05289-11

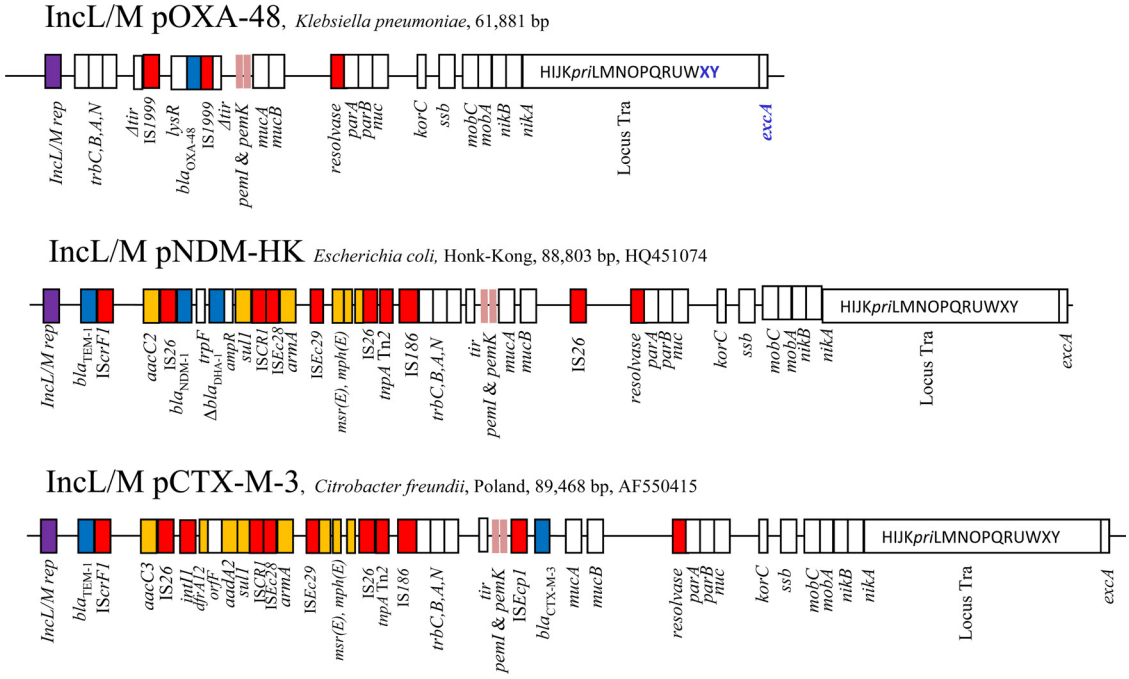

FIG 1 Major structural features of plasmid pOXA-48a from *K. pneumoniae* 11978 (22) in comparison with the IncL/M plasmids pCTX-M-3 (11) and pNDM-HK (14). White boxes indicate plasmid scaffold regions that are in common among plasmids. The locus Tra within the box is indicated. Resistance genes are indicated by orange boxes, except the β-lactamase genes, which are indicated by blue boxes. Transposon-related genes (*tnpA*, *tnpR*, and *tnpM*) and insertion sequences are indicated by red boxes. Replicase genes are indicated by purple boxes.

M-type plasmids. The other region differing from other IncL/M plasmids was located between the replication module and the *trb* transfer locus. In pCTX-M3, this 27-kb resistance island was composed of a copy of Tn*2* in which transposon Tn*1548* carried the *armA* methylase encoding gene (2). That region contained at least 19 open reading frames (ORFs), including in particular genes encoding resistance to aminoglycosides (*aadA2* and *armA*), trimethoprim (*dfrA12*), sulfonamides (*sul1*), and macrolides (*mphE* and *msrE*) (Fig. 1). In pHK-NDM, the bla_{NDM-1} gene locus was inserted within the transposon carrying the *armA* gene, replacing the class 1 integron structure identified in pCTX-M3. In pOXA-48a, no foreign DNA was inserted in the backbone at this locus.

The RepA replication protein of pOXA-48a exhibited only two amino acid differences, out of 351 amino acids, from RepA of both plasmids pCTX-M3 and pHK-NDM. Only one replication module was identified in pOXA-48a, as for other IncL/M-type plasmids known to possess broad-host-range properties that have since been identified in the *Enterobacteriaceae*, *Erwinia amylovora*, *Ralstonia eutropha*, *Pseudomonas aeruginosa*, and *Agrobacterium tumefaciens* (11, 17). Altogether, these results indicate that plasmids pOXA-48a, pCTX-M3, and pHK-NDM may have a common origin in the environment, a hypothesis which is reinforced by the identification of bla_{OXA-48}-like genes in the waterborne environmental species *S. oneidensis* and *Shewanella xiamenensis* (23, 28).

In order to better address whether plasmid pOXA-48a may possess a broad host range of replication, electrotransformation assays were carried out using DNA of pOXA-48a and different recipient species, namely, *E. coli* JM109 (control), *Acinetobacter baumannii* CIP70.10, *Pseudomonas aeruginosa* PU21, and *S. oneidensis* MR-1, with selection based on ticarcillin (50 μg/ml) (13, 29). Different protocols were used depending on the species of the recipient strain (8, 30). Whereas no transformant was obtained with *A. baumannii* and *P. aeruginosa*, pOXA-48a replicated efficiently in *E. coli* and *S. oneidensis*. This indicates that pOXA-48a possesses a broad host range of replication and that such an IncL/M-type scaffold could have been at the origin of the capture of the

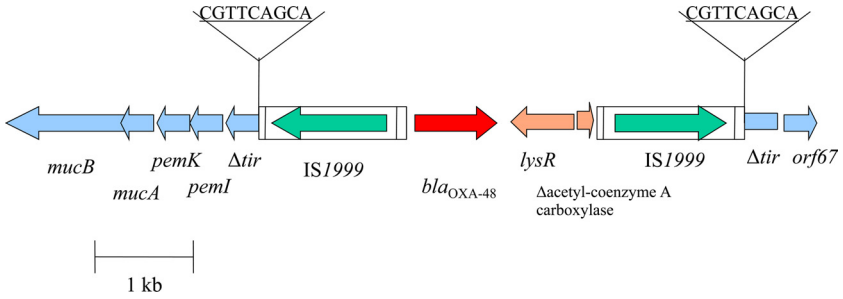

FIG 2 Genetic environment and content of Tn*1999*. The different gene names are indicated. The sequence of the 9-bp target site duplication is underlined.

TABLE 1 Primers used in this study

Primer	Sequence (5′–3′)	Reference
OXA-48A	TTGGTGGCATCGATTATCGG	22
OXA-48B	GAGCACTTCTTTTGTGATGGC	22
PBRT incL/M for	GGATGAAAACTATCAGCATCTGAAG	4
PBRT incL/M rev	CTGCAGGGGCGATTCTTTAGG	4
RepA-A	GACATTGAGTCAGTAGAAGG	This study
RepA-B	CGTGCAGTTCGTCTTTCGGC	This study
TraU-A	ATCTCACGCAATCTTACGTC	This study
TraU-B	TCGCGTCATGCGTGATCTTC	This study
ParA-A	GCAGTGAAAACGTTGATCAG	This study
ParA-B	GATCGCAATGCGTCTTGGTG	This study

bla_{OXA-48} gene from its natural progenitor (*Shewanella* spp.) and its dissemination among the *Enterobacteriaceae*. Our results also suggest that dissemination of the bla_{OXA-48} gene in *A. baumannii* or *P. aeruginosa* is unlikely to occur if it remains associated only with this type of plasmid.

The transfer operon identified in pOXA-48a was very similar to the transfer operon in pCTX-M-3, being formed by two distinct regions. This common feature suggests that the propensity for spreading of the bla_{OXA-48} gene in the *Enterobacteriaceae* might be as high as that of the $bla_{CTX-M-3}$ gene. This transfer operon included the *trb* and *tra* operons, known to encode type IV pili, which are involved in plasmid conjugation. The *tra* operon was very similar to that of pCTX-M-3, except for three genes, namely, *traX-traY-excA*, encoding proteins more distantly related (54%, 79%, and 34% identities, respectively) to those encoded by pCTX-M-3. Due to the significant divergence of this gene array, a recombination event could explain such gene array replacement. The lack of identification of pOXA-48a as an IncL/M plasmid by the PBRT method can be explained by the location of one of the corresponding primers in the *excA* gene.

In order to evaluate whether the modifications observed in pOXA-48a could influence its conjugation rate compared to that of another IncL/M-type plasmid, conjugation frequencies were determined by using as a donor the same *E. coli* J53 background harboring either pOXA-48a or pNDM-HK, along with *E. coli* JM109 as the recipient, with a selection based on ticarcillin (50 μg/ml) and nalidixic acid (20 μg/ml). No difference was observed, conjugation rates being 3.3×10^{-5} and 2.5×10^{-5} for pOXA-48a and pNDM-HK, respectively).

Since the PBRT technique was inefficient for classifying pOXA-48a, three primer pairs were newly designed to amplify conserved regions of its backbone (Table 1). They were designed in the *repA*, *traU*, and *parA* genes, encoding proteins implicated in replication, transfer, and partitioning, respectively. The presence of the bla_{OXA-48} gene was confirmed using previously designed primers (Table 1). An international collection of strains ($n = 19$) harboring the same bla_{OXA-48} gene was used for typing bla_{OXA-48}-positive plasmids. Plasmid analysis performed by using the Kieser technique (15) confirmed that they were all ca. 62 kb in size. PCR experiments confirmed that this same plasmid backbone was identified in all the bla_{OXA-48}-positive isolates (Table 2). In addition, PCR performed as described previously (17) confirmed that transposon Tn*1999* targeted the same location in all plasmids. Altogether, our results show that the current dissemination of OXA-48 producers, mostly in countries located near the Mediterranean Sea and in Western Europe, may be due largely to the spread of a single plasmid.

We report here the whole sequence of the major vehicle of the bla_{OXA-48} carbapenemase gene. Although some other widespread carbapenemase determinants, such as the bla_{KPC} and bla_{NDM} genes, have been shown to disseminate through different plasmid scaffolds (3), we showed here that the current spread of the bla_{OXA-48} gene is linked to the wide diffusion of an identical IncL/M plasmid scaffold.

Nucleotide sequence accession number. The nucleotide sequence reported in this work has been deposited in the GenBank nucleotide sequence database under accession no. JN626286.

TABLE 2 Distribution of IncL/M-type plasmids carrying the bla_{OXA-48} gene

Strain	Country	Size (kb)[a]	Plasmid feature				Plasmid-associated resistance marker	Reference
			RepA	TraU	ParA	Inc group		
K. pneumoniae 11978	Turkey	ca. 62	+	+	+	L/M	None	22
K. pneumoniae Bel	Belgium	ca. 62	+	+	+	L/M	None	5
K. pneumoniae Lib	Lebanon	ca. 62	+	+	+	L/M	None	5
K. pneumoniae 8	Turkey	ca. 62	+	+	+	L/M	None	5
K. pneumoniae Egy	Egypt	ca. 62	+	+	+	L/M	None	5
K. pneumoniae 5A	Turkey	ca. 62	+	+	+	L/M	None	5
K. pneumoniae 7A	Turkey	ca. 62	+	+	+	L/M	None	5
K. pneumoniae Bey	Lebanon	ca. 62	+	+	+	L/M	None	16
K. pneumoniae 3A	Turkey	ca. 62	+	+	+	L/M	None	5
K. pneumoniae 4A	Turkey	ca. 62	+	+	+	L/M	None	5
K. pneumoniae 17A	Turkey	ca. 62	+	+	+	L/M	None	5
K. pneumoniae Bou	France	ca. 62	+	+	+	L/M	None	10
K. pneumoniae Dia	France	ca. 62	+	+	+	L/M	None	9
K. pneumoniae C2	Morocco	ca. 62	+	+	+	L/M	None	Unpublished
K. oxytoca A7	Morocco	ca. 62	+	+	+	L/M	None	Unpublished
E. coli 1	Turkey	ca. 62	+	+	+	L/M	None	5
E. cloacae 501	France	ca. 62	+	+	+	L/M	None	25
E. cloacae Bou	Morocco	ca. 62	+	+	+	L/M	None	25
E. cloacae D4	Morocco	ca. 62	+	+	+	L/M	None	Unpublished

[a] Sizes were often reported to be ca. 70 kb according to the size markers used, but present comparison with plasmid pOXA-48 from *K. pneumoniae* 11978 revealed the same size.

ACKNOWLEDGMENT

This work was funded by a grant from the INSERM (U914).

REFERENCES

1. **Aubert D, Naas T, Héritier C, Poirel L, Nordmann P.** 2006. Functional characterization of IS*1999*, an IS*4* family element involved in mobilization and expression of β-lactam resistance genes. J. Bacteriol. **188**:6506–6514.
2. **Bailey JK, Pinyon JL, Anantham S, Hall RM.** 2011. Distribution of the blaTEM gene and blaTEM-containing transposons in commensal *Escherichia coli.* J. Antimicrob. Chemother. **66**:745–751.
3. **Carattoli A.** 2009. Resistance plasmid families in *Enterobacteriaceae.* Antimicrob. Agents Chemother. **53**:2227–2238.
4. **Carattoli A, et al.** 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods **63**:219–228.
5. **Carrër A, et al.** 2008. Spread of OXA-48-positive carbapenem-resistant *Klebsiella pneumoniae* isolates in Istanbul, Turkey. Antimicrob. Agents Chemother. **52**:2950–2954.
6. **Carrër A, et al.** 2010. Spread of OXA-48-encoding plasmid in Turkey and beyond. Antimicrob. Agents Chemother. **54**:1369–1373.
7. **Castanheira M, et al.** 2011. Early dissemination of NDM-1- and OXA-181-producing *Enterobacteriaceae* in Indian hospitals: report from the SENTRY antimicrobial surveillance program, 2006–2007. Antimicrob. Agents Chemother. **55**:1274–1278.
8. **Choi KH, Kumar A, Schweizer HP.** 2006. A 10-min method for preparation of highly electrocompetent *Pseudomonas aeruginosa* cells: application for DNA fragment transfer between chromosomes and plasmid transformation. J. Microbiol. Methods **64**:391–397.
9. **Cuzon G, Naas T, Lesenne A, Benhamou M, Nordmann P.** 2010. Plasmid-mediated carbapenem-hydrolysing OXA-48 β-lactamase in Klebsiella pneumoniae from Tunisia. Int. J. Antimicrob. Agents **36**:91–93.
10. **Cuzon G, Ouanich J, Gondret R, Naas T, Nordmann P.** 2011. Outbreak of OXA-48-positive carbapenem-resistant *Klebsiella pneumoniae* in France. Antimicrob. Agents Chemother. **55**:2420–2423.
11. **Golebiewski M, et al.** 2007. Complete nucleotide sequence of the pCTX-M3 plasmid and its involvement in spread of the extended-spectrum β-lactamase gene $bla_{\text{CTX-M-3}}$. Antimicrob. Agents Chemother. **51**:3789–3795.
12. **Goren MG, Chmelnitsky I, Carmeli Y, Navon-Venezia S.** 2011. Plasmid-encoded OXA-48 carbapenemase in *Escherichia coli* from Israel. J. Antimicrob. Chemother. **66**:672–673.
13. **Heidelberg JF, et al.** 2002. Genome sequence of the dissimilatory metal ion-reducing bacterium *Shewanella oneidensis.* Nat. Biotechnol. **20**:1118–1123.
14. **Ho PL, et al.** 2011. Complete sequencing of pNDM-HK encoding NDM-1 carbapenemase from a multidrug-resistant *Escherichia coli* strain isolated in Hong Kong. PLoS One **6**:e17989.
15. **Kieser T.** 1984. Factors affecting the isolation of CCC DNA from *Streptomyces lividans* and *Escherichia coli.* Plasmid **12**:19–36.
16. **Matar GM, et al.** 2008. Oxacillinase-mediated resistance to carbapenems in *Klebsiella pneumoniae* from Lebanon. Clin. Microbiol. Infect. **14**:887–888.
17. **Mierzejewska J, Kulińska A, Jagura-Burdzy G.** 2007. Functional analysis of replication and stability regions of broad-host-range conjugative plasmid CTX-M3 from the IncL/M incompatibility group. Plasmid **57**:95–107.
18. **Moquet O, et al.** 2011. Class D OXA-48 carbapenemase in multidrug-resistant enterobacteria, Senegal. Emerg. Infect. Dis. **17**:143–144.
19. **Poirel L, Bonnin RA, Nordmann P.** Genetic basis of antibiotic resistance in pathogenic *Acinetobacter* species. IUBMB Life, in press.
20. Reference deleted.
21. **Poirel L, et al.** 2011. OXA-163, an OXA-48-related class D β-lactamase with extended activity toward expanded-spectrum cephalosporins. Antimicrob. Agents Chemother. **55**:2546–2551.
22. **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.
23. **Poirel L, Naas T, Nordmann PP.** 2010. Diversity, epidemiology, and genetics of class D β-lactamases. Antimicrob. Agents Chemother. **54**:24–38.
24. **Poirel L, Pitout JD, Nordmann P.** 2007. Carbapenemases: molecular diversity and clinical consequences. Future Microbiol. **2**:501–512.
25. **Poirel L, et al.** 2011. Cross-border transmission of OXA-48-producing *Enterobacter cloacae* from Morocco to France. J. Antimicrob. Chemother. **66**:1181–1182.
26. **Poirel L, et al.** 2011. Extremely drug-resistant *Citrobacter freundii* isolate producing NDM-1 and other carbapenemases identified in a patient returning from India. Antimicrob. Agents Chemother. **55**:447–448.
27. **Potron A, et al.** 2011. Characterization of OXA-181, a carbapenem-hydrolyzing class D β-lactamase from *Klebsiella pneumoniae.* Antimicrob. Agents. Chemother. **55**:4896–4899.
28. **Potron A, Poirel L, Nordmann P.** 2011. Origin of OXA-181, an emerging carbapenem-hydrolyzing oxacillinase, as a chromosomal gene in *Shewanella xiamenensis.* Antimicrob. Agents Chemother. **55**:4405–4407.
29. **Potron A, Poirel L, Nordmann P.** 2011. Plasmid-mediated transfer of the $bla_{\text{NDM-1}}$ gene in Gram-negative rods. FEMS Microbiol. Lett. **324**:111–116.
30. **Sambrook J, Fritsch EF, Maniatis T.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.