Complete Sequencing of Plasmids Containing *bla*_{OXA-163} and *bla*_{OXA-48} in *Escherichia coli* Sequence Type 131

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OXA-48-like enzymes have emerged as important extended-spectrum β -lactamases/carbapenemases in *Escherichia coli* sequence type 131 (ST131). We report the structures of the first fully sequenced *bla*_{OXA-163} plasmid and of two other *bla*_{OXA-48} plasmids in this lineage. *bla*_{OXA-163} was located on a 71-kb IncN plasmid with other resistance genes. *bla*_{OXA-48} was present on IncL/M plasmids, genetically similar to other *bla*_{OXA-48} plasmid sequences, and consistent with interspecies/interlineage spread. The presence of *bla*_{OXA-48}-like genes on epidemic plasmids in ST131 is of concern.

The emergence of transmissible carbapenemases in successful clinical lineages of *Escherichia coli*, such as multilocus sequence type 131 (ST131), is a potential health care threat (1). The OXA-48-like enzymes (including OXA-48, OXA-162, OXA-163, OXA-181, OXA-204, and OXA-232) represent one of the major families responsible for extended-spectrum beta-lactam/carbapenem resistance in *Enterobacteriaceae* (2). Here we report the genomes and plasmid sequences of a highly drug-resistant *bla*_{OXA-163}-harboring *E. coli* ST131 isolate (Ecol_448) and of two *bla*_{OXA-48} *E. coli* ST131 isolates (Ecol_743 and Ecol_745) in order to understand the genetic structures associated with *bla*_{OXA-163} and *bla*_{OXA-48} in ST131.

Isolates Ecol_448, Ecol_743, and Ecol_745 were identified as part of the Merck Study for Monitoring Antimicrobial Resistance Trends (SMART) global surveillance of antimicrobial resistance described previously (Table 1) (3). All isolates were cultured from urine specimens from hospitalized, infected patients. MICs to ampicillin-sulbactam (SAM), piperacillin-tazobactam (TZP), cefoxitin (FOX), ceftriaxone (CRO), ceftazidime (CAZ), cefepime (FEP), ertapenem (ERT), imipenem (IPM), amikacin (AMK), and ciprofloxacin (CIP) were determined using microdilution panels (International Health Management Associates, Inc., Schaumburg, IL, USA) following 2015 CLSI guidelines.

DNA was extracted from pure isolate subcultures using the Qiagen genomic tip 100/G and QIAamp DNA minikits (Qiagen, Valencia, CA) for PacBio (long-read) sequencing and Illumina MiSeq (short-read) sequencing, respectively. PacBio sequencing and preliminary *de novo* assembly using HGAP3 were performed as previously described (4). PacBio contigs were annotated using PROKKA (5) and trimmed in Geneious (version 9.04) (6) on the basis of sequence/annotation overlaps. The 150-base, paired-end MiSeq reads for each isolate were trimmed using Trimmomatic (version 0.35) (7) and mapped to the corresponding PacBio assemblies using BWA mem (version 0.7.9a-r786) (8). Unmapped MiSeq reads were *de novo* assembled using A5-MiSeq (9) to capture small plasmids (<7 kb) potentially filtered out in the size selection process during PacBio library preparation. The mean coverage/percent nucleotide consensus values following mapping

of the MiSeq reads to the trimmed PacBio assemblies were 112.3/99.38% for isolate Ecol_743, 92.5/99.35% for isolate Ecol_745, and 74.2/99.3% for isolate Ecol_448. Read pileups were inspected to confirm structural integrity of the PacBio contigs, and small errors were corrected manually in line with the pileup consensus. PROKKA annotations were refined with reference to the ISFinder, ResFinder, and in-house resistance gene databases; *in silico* Inc typing was performed using PlasmidFinder (10–13).

To generate comparisons with other fully sequenced plasmids carrying *bla*_{OXA-48} or *bla*_{OXA-163}, we searched the NCBI nucleotide database (<http://www.ncbi.nlm.nih.gov/nucleotide>) using the following terms: “OXA-48 [All Fields] and (“unidentified plasmid” [Organism] or plasmid [All Fields])” and “OXA-163 [All Fields]” (date of search, 12 March 2016). Results were filtered to exclude partial plasmid sequences, those that did not contain *bla*_{OXA-48} or *bla*_{OXA-163}, and those that represented duplicates of the same plasmid sequence (leaving 10 plasmids for analysis). Plasmid sequences were initially compared using a BLASTn-based, pairwise assessment of sequence homology to generate an average similarity metric for each plasmid pair (scripts available at https://github.com/aesheppard/plasmid_comp). Complete sequence alignments were then undertaken for pOXA48 (as the earliest available sequence), the respective study plasmids, and the most similar publicly available plasmids identified from the similarity metrics (scores of $\geq 90\%$ for Fig. 1A and $\geq 99\%$ for Fig. 1B), using MUSCLE (plug-in in Geneious, default settings; iterations capped at six) (6).

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TABLE 1 Details of publicly available *bla*_{OXA-48} and *bla*_{OXA-48-like} plasmids identified in this study

Plasmid	GenBank accession no.	Geographic origin	Yr of isolation	Species isolated from	Size (bp)	Inc type	Genetic context of <i>bla</i> _{OXA}
pOXA-48	JN626286	Istanbul, Turkey	2001	<i>Klebsiella pneumoniae</i>	61,881	L/M	Tn1999.1
pRA35	LN864821	Tripoli, Lebanon	2011	<i>Raoultella planticola</i>	63,434	L/M	Inverted Tn1999.2
pKP112	LN864819			<i>Klebsiella pneumoniae</i>	84,252	L/M	Inverted Tn1999.2
pOXA-48E1	KP659188			<i>Klebsiella pneumoniae</i>	62,014	L/M	Inverted Tn1999.2
pCF29	LN864820			<i>Citrobacter freundii</i>	49,151	L/M	Inverted Tn1999.2
pE71T	KC335143	Ireland		<i>Klebsiella pneumoniae</i>	63,578	L/M	Tn1999.2
pKPN-E1.Nr7	KM406491	Bern, Switzerland (transfer from Belgrade, Serbia)	2013	<i>Klebsiella pneumoniae</i>	63,581	L/M	Tn1999.2
pKPoxa-48N1	KC757416	Nancy, France	2010	<i>Klebsiella pneumoniae</i>	62,592	L/M	Tn1999.2
pKPoxa-48N2	KC757417	Nancy, France	2010	<i>Klebsiella pneumoniae</i>	167,203	L/M	Tn1999.2
pJEG011	KC354801	Sydney, Australia (patient recently returned from Egypt)	2010	<i>Klebsiella pneumoniae</i>	71,446	L/M	Tn1999.1
pOXA48-Pm	KP025948	Gaza, Palestine	2012	<i>Proteus mirabilis</i>	72,127	L/M	Inverted Tn1999.2
pEC743-OXA48 (this study)	CP015071.1	Dubai, United Arab Emirates	2012	<i>Escherichia coli</i>	69,471	L/M	Tn1999.2
pEC745-OXA48 (this study)	CP015075.1	Rabat, Morocco	2012	<i>Escherichia coli</i>	63,543	L/M	Tn1999.2
pEC448-OXA163 (this study)	CP015078.1	Buenos Aires, Argentina	2011	<i>Escherichia coli</i>	71,171	N2	Δ IS1326-ISShfr9-Tn3-IS4321- <i>bla</i> _{OXA-163} -IS6100

The MICs for each isolate to IPM and ERT (in milligrams/liter) were as follows: 0.5 and 0.5 for isolate Ecol_743, respectively; 1 and 1 for isolate Ecol_745, respectively; and 0.25 and 0.12 for isolate Ecol_448, respectively. All isolates were nonsusceptible (i.e., either intermediate or resistant) to SAM, TZP, and CIP. Isolate Ecol_743 was also nonsusceptible to CTX, CRO, and AMK. Isolate Ecol_745 was nonsusceptible to AMK, and isolate Ecol_448 was nonsusceptible to FOX, FEP, CTX, and CRO.

The Ecol_743 genome consisted of a chromosome (4,856,574 bp) and four plasmids (111,851 bp, 69,471 bp [harboring *bla*_{OXA-48}; pEC743-OXA48], 65,196 bp, and 37,000 bp). The Ecol_745 genome consisted of a chromosome (4,977,366 bp) and a single plasmid harboring *bla*_{OXA-48} (63,544 bp, pEC745-OXA48). The Ecol_448 genome consisted of a chromosome (5,007,393 bp) and two plasmids (133,740 bp and 71,171 bp [harboring *bla*_{OXA-163}; pEC448-OXA163]). Details of relevant *bla*_{OXA-48} plasmid sequences from GenBank and the *bla*_{OXA} plasmids identified in this study are summarized in Table 1; there were no fully sequenced *bla*_{OXA-163} plasmids available for comparison.

pEC448-OXA163 is an IncN2 plasmid, with only partial homology to several other (non-OXA) carbapenemase-harboring plasmids in GenBank (top BLASTn match by query cover [49%] and identity [99%] was p0801-IMP [containing *bla*_{IMP-1}; GenBank accession number [KT345947.1](#)]). The *bla*_{OXA-163} gene itself is located within a complex cache of transposable elements similar, but not identical, to those found in two *Klebsiella pneumoniae* and *Enterobacter cloacae* strains isolated in Argentina in 2008. In these two cases, the plasmid backbones were approximately sized (70 kb and 85 kb, respectively) but not further characterized, and their incompatibility groups could not be ascertained by PCR-based replicon typing (14). The genetic context of *bla*_{OXA-163} in reference 14 was explored only with partial sequencing of the two isolates, demonstrating similar but distinct flanking motifs: in the *E. cloacae* plasmid (pEnc2185), *bla*_{OXA-163} was located between an IS4321 Δ Tn3-like element and a Δ IS4 IS1600 element; in the *K. pneumoniae* plasmid (pKp6299), it was located between an IS4321 Δ Tn3-like element and an intact IS4-like ele-

ment. In pEC448-OXA163, *bla*_{OXA-163} is located between an IS4321 element and an intact IS1600 element, as in pEnc2185, but downstream of a Δ IS1236 ISShfr9 unit and a Tn3 unit harboring *bla*_{OXA-9}, *aadA1*, *aac(6')*-Ib-cr, and *bla*_{TEM-1}. Additional resistance genes identified in pEC448-OXA163 include *dfrA16*, *sul1*, and *qnrB10*.

pEC743-OXA48, an IncL/M plasmid, is most similar to pOXA48-Pm (GenBank accession number [KP025948](#); 97.1% average similarity index; $\leq$ 92% for all other *bla*_{OXA-48} plasmids). However, in pEC743-OXA48, *bla*_{OXA-48} is within Tn1999.2, and in pOXA48-Pm, it is located in an inverted Tn1999.2 element, consistent with its inversion within this plasmid backbone. In addition, substantial nucleotide level variation is present in the *rep* gene region (pairwise percentage identity of 94.3%), which is flanked by identical sequence, consistent with a homologous recombination event. The other differences were 11 single nucleotide variants and three likely insertion/deletion events, represented by the presence of ISEcp1-*bla*_{CTX-M-14} (3,100 bp) in pOXA48-Pm and two unannotated stretches of sequence in pOXA48-Pm (335 bp) and pEC743-OXA48 (777 bp), respectively (Fig. 1A). Only a few other resistance genes were present, all of which confer resistance to aminoglycosides, namely, *strA*, *strB*, and *aph(3')*-Vib (15). pEC743-OXA48, pOXA48-Pm, and pJEG011 (from *K. pneumoniae* in Australia) have most likely evolved from pOXA-48 through several recombination and insertion deletion events affecting the *rep-relB* region (Fig. 1A).

pEC745-OXA48, also of the IncL/M group, is highly genetically and structurally similar to the six other publicly available OXA-48 plasmids of the same size ($\sim$ 63 kb; all $\geq$ 98%; Table 1) (Fig. 1B). In addition, it shows high similarity to much of pKP112 (this IncL/M plasmid background with a 20,676-bp Tn21-like element inserted) and pKPoxa-48N2 (a hybrid of this plasmid background with pEC-IMP and pc-15k [16]) and pCF29 (which has a deletion of the *traK-IS1R* region relative to pOXA-48). As with these reference plasmids, no other known resistance genes were present in pEC745-OXA48.

OXA-163 has a greater capacity to hydrolyze extended-spec-

FIG 1 Modified alignments of pOXA-48, pEC743-OXA48 (this study; shown in red), and pEC745-OXA48 (this study; shown in red), alongside other closely related publicly available plasmid sequences. (A) Plasmids pOXA48, pJEG011, POXA48-Pm, and pEC743-OXA48. (B) Plasmids pOXA48, pE71T, pKPoxa-48N1, pKpn-E1.Nr7, pRA35, and pEC745-OXA48. For each comparison, in the sequence bars, gray regions represent regions of 100% sequence identity with respect to the reference pOXA-48 sequence. The reference pOXA-48 sequence is shown all in black on a pale yellow background. Black regions in nonreference sequence represent genetically divergent regions. Annotations with the same color indicate high similarity (≥ 99.5 sequence identity) among subsets of sequences. Yellow annotations are those that are genetically highly similar in all sequences within the comparison (99.5 to 100% sequence identity). Other features of interest are colored as shown in the color legend in panel A; *repA* and *relB* are annotated above the sequence bar. Numbering reflects the alignment column number.

trum cephalosporins and lower capacity to hydrolyze carbapenems compared to the other enzymes in the OXA-48 group (14), and therefore, it may not be recognized in routine phenotypic surveillance. Its activity against carbapenems may be enhanced in the presence of permeability defects (17). Its location on an IncN plasmid with multiple other resistance mechanisms is of concern, as this is one of several plasmid families that have played a significant role in the dissemination of other problematic extended-spectrum beta-lactamases/carbapenemases in *E. coli* ST131 (18). OXA-163 is potentially endemic in hospitals in Buenos Aires, Argentina, and has been reported in Cairo, Egypt (17); given the difficulties in identifying it through phenotypic surveillance, it may be more widespread than previously thought.

In conclusion, this study is the first to report complete *bla*_{OXA-48-like} plasmid sequences from *E. coli* ST131. We have characterized the first complete sequence of a *bla*_{OXA-163} plasmid thus far and demonstrated that an epidemic IncL/M-*bla*_{OXA-48} plasmid has disseminated to *E. coli* ST131, as previously hypothesized in several studies in Spain, United Kingdom, and Ireland (19–21).

Accession number(s). Complete sequence data for all isolates have been deposited in GenBank under the BioProject accession number [PRJNA316786](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA316786). Accession numbers for the specific plasmid sequences described in this study are listed in [Table 1](#).

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