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First Description of OXA-48 Carbapenemase Harbored by *Escherichia coli* and *Enterobacter cloacae* from a Single Patient in Portugal

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The bla_{OXA-48} gene has been detected in a variety of species and clones of *Enterobacteriaceae*, circulating mainly throughout the Mediterranean area but progressively disseminating to other geographical areas (1, 2). To our knowledge, we describe here the first cases of OXA-48-producing *Enterobacteriaceae* in Portugal.

Enterobacter cloacae and *Escherichia coli* were isolated from the urine of a catheterized patient presenting with pyuria, hematuria, and positive nitrites. The patient was a 74-year-old Caucasian female, admitted at the emergency room of a hospital in Lisbon, Portugal, in March 2013, who was diagnosed with decompensated heart failure and who had been previously treated with the combination amoxicillin-clavulanate, due to infected leg ulcers. In the patient's clinical history, we noted a left nephrectomy for oncocytoma, a nonfunctional right adrenal adenoma, the use of medication for high blood pressure, and several previous admissions for cardiac decompensation.

The two isolates were sent to the National Institute of Health due to their carbapenem resistance, where the genotype was studied to determine the main resistance mechanisms. By the microdilution method, both isolates were found to be nonsusceptible to different classes of antibiotics, with susceptibility to colistin and tigecycline (http://www.eucast.org/clinical_breakpoints/) (Table 1). Conjugation experiments were performed using *E. coli* J53 (sodium azide resistant) as the recipient, as previously described (3). *E. coli* J53 Tc17313-1 and *E. coli* J53 Tc17313-2 transconjugants were resistant to penicillins and ertapenem but remained susceptible to oximino-β-lactams; both transconjugants were additionally nonsusceptible to meropenem and gentamicin, respectively (Table 1).

PCR amplification and sequencing of the most prevalent β-lactamase- and plasmid-mediated quinolone resistance (PMQR)-encoding genes, performed as previously described (3), allowed the identification of the bla_{OXA-48} gene in both isolates and transconjugants; the chromosomal bla_{ACT-34} variant and the *qnrD* genes were detected in

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TABLE 1 Phenotypic and genotypic context of OXA-48-producing clinical isolates, transconjugants, and the recipient strain[a]

Antimicrobial drug[b]	MIC (μg/ml) for strain:				
	E. coli J53	*E. cloacae* INSRA17313-1 (OXA-48, ACT-34)	*E. coli* J53 Tc17313-1 (OXA-48)	*E. coli* INSRA17313-2 (OXA-48, QnrD)	*E. coli* J53 Tc17313-2 (OXA-48)
Amoxicillin	4	>256	>256	>256	>256
Amoxicillin-clavulanate	4	256	64	128	64
Ticarcillin	≤4	1,024	128	512	256
Cefuroxime	2	512	4	8	4
Cefoxitine	8	512	8	16	8
Ceftazidime	≤0.5	64	≤0.5	4	≤0.5
Ceftazidime-clavulanate	≤0.5	16	≤0.5	≤0.5	≤0.5
Cefotaxime	≤0.5	512	2	2	1
Cefotaxime-clavulanate	≤0.5	256	1	2	≤0.5
Aztreonam	≤0.5	128	≤0.5	≤0.5	≤0.5
Cefepime	≤1	1,024	≤1	16	≤1
Imipenem	≤0.5	4	1	1	1
Meropenem	≤0.125	8	4	2	2
Doripenem	≤0.125	2	≤0.125	≤0.125	≤0.125
Ertapenem	≤0.06	>64	4	4	2
Ciprofloxacin	≤0.06	2	≤0.06	≤0.06	≤0.06
Gentamicin	2	32	2	8	8
Trimethoprim	≤2	>4,096	≤2	8	≤2
Colistin	0.5	1	0.25	0.25	0.25
Tigecycline	0.25	0.5	0.125	0.125	0.125

[a] *E. coli* J53 Tc17313-1 (harboring OXA-48) and *E. coli* J53 Tc17313-2 (harboring OXA-48) were transconjugants of *E. cloacae* INSRA17313-1 (harboring OXA-48 and ACT-34) and *E. coli* INSRA17313-2 (harboring OXA-48 and QnrD), respectively; *E. coli* J53 was the recipient strain.
[b] The combinations with clavulanate included that drug at 2 μg/ml.

E. cloacae and *E. coli* clinical isolates, respectively (Table 1). This is the first description of QnrD-producing *E. coli* in Europe (4).

By PCR mapping (5), we confirmed the presence of a Tn*1999.2* structure containing the bla_{OXA-48} gene. This composite transposon, located in a conjugative plasmid, encompassed two copies of the insertion sequence IS*1999*, with IS*1R* truncating the left-hand copy of the transposase. This Tn*1999.2* structure has been described to be associated with higher carbapenem MICs than Tn*1999.1* (5, 6). Indeed, the bla_{OXA-48} gene is usually part of Tn*1999* and located in a plasmid (6, 7), although a chromosome-mediated bla_{OXA-48} gene has recently been found in *E. coli* (8).

None of the plasmids carried by the clinical isolates and transconjugants belonged to an incompatibility group identified by a PCR-based replicon typing method (9). However, we detected, as previously described (10), the presence of the *repA*, *traU*, and *parA* genes, suggesting that those plasmids had an IncL/IncM-pOXA-48a-like backbone (10). The OXA-48-producing *E. coli* isolate belonged to the ST1429 lineage, based on multilocus sequence typing (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). This sequence type was previously reported in Sweden in 2004 in an *E. coli* isolate that did not produce any carbapenemase (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli).

In conclusion, this study shows the presence of OXA-48-producing *Enterobacteriaceae* in Portugal. Although we could not link our patient with a history of travel to countries of endemicity and no other case was identified in that hospital, we highlight the potential of interspecies dissemination of bla_{OXA-48} gene-harboring plasmids and, consequently, the importance of concerted actions to manage carbapenem resistance.

Nucleotide sequence accession number. The new bla_{ACT} nucleotide sequence was submitted to the EMBL nucleotide sequence database as bla_{ACT-34} under accession number HG975300.

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