Escherichia coli from Italy Producing OXA-48 Carbapenemase Encoded by a Novel Tn*1999* Transposon Derivative

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OXA-48 is an emerging class D carbapenemase originally identified in isolates from Turkey (14) and subsequently detected in several European and north African countries (10). *Klebsiella pneumoniae* is the most common host for OXA-48, but the enzyme has also been detected in *Escherichia coli* and *Enterobacter cloacae* (10). The bla_{OXA-48} gene is carried by the composite transposon Tn*1999* or a variant thereof, named Tn*1999.2* (1), and is plasmid mediated. A 60-kb IncL/M-related conjugative plasmid, named pOXA-48a, which carries the carbapenemase gene as the only resistance determinant, was found to mediate the horizontal dissemination of bla_{OXA-48} among strains of different species and from different sources (13).

In this report we describe the first case of a strain from Italy producing OXA-48, encoded by a pOXA-48a-like plasmid which carries a new variant of Tn*1999*.

E. coli ECBZ-1 was isolated in April 2011 from a urinary tract infection (UTI) of a 54-year-old female patient living in northern Italy. Susceptibility testing, performed by Etest (bioMérieux, Marcy l'Etoile, France), revealed that the isolate was resistant to trimethoprim-sulfamethoxazole, penicillins (including β-lactamase inhibitor combinations), narrow-spectrum cephalosporins, and ertapenem (even by disk diffusion), while it remained susceptible to other carbapenems, expanded-spectrum cephalosporins, aminoglycosides, fluoroquinolones, and fosfomycin (Table 1). Initially treated empirically with trimethoprim-sulfamethoxazole without improvement, the infection was successfully treated with oral fosfomycin (3 g per day for 2 days). A modified Hodge test (9) suggested production of carbapenemase, but neither EDTA nor boronic acid affected meropenem susceptibility in combination disk tests (16). PCR analysis (3, 4, 6, 11, 12, 14), and sequencing revealed the presence of a bla_{OXA-48} carbapenemase gene and of a bla_{TEM-1b} gene. This β-lactamase profile was consistent overall with the resistance phenotype of ECBZ-1, since OXA-48 hydrolyzes carbapenems (although weakly), penicillins, and narrow-spectrum cephalosporins but not expanded-spectrum cephalosporins and is not inhibited by clavulanate and sulfones (5, 14). The peculiar resistance phenotype of ECBZ-1 underlines the importance of surveillance of isolates showing resistance only to ertapenem even in the presence of susceptibility to expanded-spectrum cephalosporins.

Analysis of clinical records revealed a history of recurrent UTIs and a previous vacation (in November 2010) in Egypt, a country where OXA-48-producing isolates have been reported (10), suggesting a cross-border source for the OXA-48-producing strain.

ECBZ-1 belongs in phylogenetic group D (2) and in sequence type ST2076 (http://mlst.ucc.ie/mlst/dbs/Ecoli) related to clonal complex 394, which also includes ST471, which was found to be associated with diffusion of KPC-2 carbapenemase in Israel (7).

A conjugation experiment (8) using *E. coli* J53 (*pro met* Rif^r Nal^r) (17) as the recipient and selection with ertapenem (0.5 μg/ml), nalidixic acid (32 μg/ml), and rifampin (250 μg/ml) yielded J53 transconjugants (frequency, $7 \times 10^{-5} \pm 1.5 \times 10^{-5}$ transconjugants per recipient) containing the bla_{OXA-48} but not the bla_{TEM-1b} gene, as assessed by PCR, and showing a resistance phenotype resembling that of the donor except for resistance to trimethoprim-sulfamethoxazole (Table 1). Analysis of the plasmid (15) from a randomly selected transconjugant revealed a ca. 60-kb plasmid, named pECBZ-1 (data not shown), whose backbone was confirmed to be identical or closely related to that of the pOXA-48a IncL/M type plasmid by PCR analysis of the *repA*, *traU*, and *parA* genes (13).

TABLE 1 MICs for *E. coli* ECBZ-1, J53(pECBZ-1), and J53

Antibiotic	MIC (μg/ml) (category)[a]		
	ECBZ-1	J53 (pECBZ-1)	J53
Ampicillin	>32 (R)	>32	0.5
Amoxicillin-clavulanate	>32 (R)	>32	0.5
Cefotaxime	0.75 (S)	0.75	0.25
Ceftazidime	1 (S)	0.38	0.25
Cefepime	0.75 (S)	0.125	0.047
Imipenem	1[b] (S)	0.5	0.064
Ertapenem	2[b] (R)	1	0.016
Meropenem	1[b] (S)	0.5	0.008
Piperacillin-tazobactam	256 (R)	8	0.25
Levofloxacin	0.064 (S)	ND	ND
Ciprofloxacin	0.023 (S)	ND	ND
Amikacin	2 (S)	ND	ND
Gentamicin	0.064 (S)	ND	ND
Tobramycin	1 (S)	ND	ND
Trimethoprim-sulfamethoxazole	256 (R)	0.023	0.023
Tigecycline	0.75 (S)	ND	ND
Colistin	0.19 (S)	ND	ND

[a] MICs were determined for *E. coli* ECBZ-1, *E. coli* transconjugant J53(pECBZ-1), and recipient strain *E. coli* J53. Category classification was based on updated EUCAST breakpoints (http://www.eucast.org/clinical_breakpoints/ [version 2.0, January 1 2012]). R, resistant; S, susceptible; ND, not determined.
[b] Diameters of inhibition: imipenem, 23 mm (S); meropenem, 22 mm (S); ertapenem, 16 mm (R).

Mapping of the bla_{OXA-48}-flanking regions and sequencing of the amplicons revealed an original genetic context that was similar overall to Tn*1999.2* (1) but differed from the latter by the presence of a second copy of IS*1R* inserted 199 bp downstream bla_{OXA-48} (Fig. 1). In this new Tn*1999* transposon variant, named Tn*1999.3*, bla_{OXA-48} could have the advantage of overexpression driven by the upstream IS*1R* copy (as in Tn*1999.2* [1]), while the two copies of IS*1R* flanking bla_{OXA-48} define a new putative composite transposon that might further mobilize the carbapenemase gene (Fig. 1). Mapping of the Tn*1999.3*-flanking regions revealed that the transposon was inserted into a *tir* gene, at the same position re-

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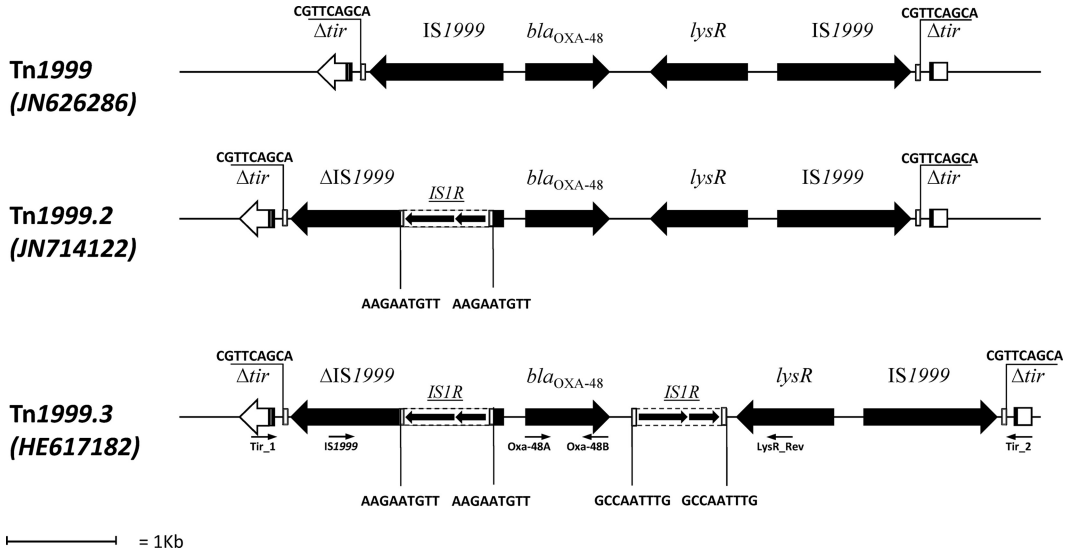

FIG 1 Linear map of the transposon Tn*1999.3* carried by ECBZ-1, compared with the other Tn*1999* variants. Small arrows show primer positions. Primers used to characterize Tn*1999.3* were OXA-48A and OXA-48B (14), IS1999 (5′-TGATGTTGTGCTTGGTTCGG-3′), LysR_Rev (5′-GCTAGTGCCAATCTTACAGG-3′), Tir_1 (5′-GCCCAACAGTAAACCCAGC-3′), and Tir_2 (5′-AGTTTATGCTGGTTCTGCTGG-3′). The putative composite transposon formed by the two IS*1R* insertion sequences, carrying bla_{OXA-48}, is underlined.

ported for Tn*1999* in plasmid pOXA-48a (13) (Fig. 1), suggesting that that Tn*1999.3* evolved from Tn*1999* by stepwise insertion of the IS*1R* elements into the latter transposon, once it had been incorporated into the plasmid backbone. This hypothesis for the evolution of Tn*1999.3* is also consistent with the fact that no direct repeats are present flanking the putative composite transposon made up of the two IS*1R* elements.

Nucleotide sequence accession number. The nucleotide sequence of the bla_{OXA-48} gene and flanking regions has been submitted to the GenBank/EMBL database and assigned accession no. HE617182.

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