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## Characterisation of OXA-244, a chromosomally-encoded OXA-48-like β-lactamase from *Escherichia coli*

*Sir,*

During the last decade, the carbapenem-hydrolysing β-lactamase OXA-48 has rapidly and widely disseminated, now being the most commonly identified carbapenemase in most European and Mediterranean countries [1]. Since its discovery, ten variants of OXA-48 have been reported [1]. In most cases, $bla_{\text{OXA-48-like}}$ genes are plasmid-borne and have been identified associated with insertion sequences involved in their acquisition and expression [1,2].

*Escherichia coli* strain VAL was recovered from a urine sample of an 85-year-old patient with no history of travel abroad. The isolate was resistant to penicillins and penicillin/β-lactamase inhibitor combinations but remained susceptible to broad-spectrum cephalosporins, imipenem and meropenem, being of intermediate susceptibility to ertapenem. Multilocus sequence typing (MLST) showed that *E. coli* VAL belonged to sequence type ST38 [1], known to be a successful international clone [3]. Using PCR experiments followed by sequencing [2], *E. coli* VAL was found to harbour a $bla_{\text{OXA-48-like}}$ gene, termed $bla_{\text{OXA-244}}$ (http://www.lahey.org/studies/). Compared with OXA-48, OXA-244 exhibits a single Arg214Gln substitution. To compare the hydrolytic profile of OXA-244 with that of OXA-48, the corresponding genes were cloned into the vector pCR-BluntII-TOPO as described previously [2] and were expressed in *E. coli* HB4, which lacks porins OmpF and OmpC. Expression of both carbapenemase genes conferred high-level resistance to imipenem, meropenem and ertapenem [minimum inhibitory concentrations (MICs) ≥ 32 mg/L]. Noteworthy, the MICs of imipenem and temocillin were lower for OXA-244 (32 mg/L and 96 mg/L, respectively) than those for OXA-48 (>32 mg/L and >1024 mg/L, respectively), suggesting a weaker activity towards these substrates for OXA-244. Similar results have been observed with OXA-232, differing from OXA-181 by a single amino acid substitution at position 214, which is located near the active site of the enzyme [2]. These two examples highlight the importance of the integrity of this residue at position 214 in the hydrolytic capacities of OXA-48-like β-lactamases. Specific activities of OXA-244 for ertapenem and meropenem (2.2 and 4.3 mU/mg of protein, respectively) were close to those of OXA-48 (3.5 and 5.4 mU/mg of protein, respectively) [1]. However, the specific activity of OXA-244 for imipenem (4.1 mU/mg of protein) was much lower than that determined for OXA-48 (111 mU/mg of protein), showing a weak hydrolysis of imipenem by OXA-244. Plasmid DNA analysis showed that *E. coli* VAL harboured three plasmids of 120, 80 and 10 kb (data not shown). Despite several attempts, no electrotransformant or transconjugant could be obtained, suggesting a chromosomal location of the $bla_{\text{OXA-244}}$ gene. The genetic environment of the $bla_{\text{OXA-244}}$ gene was determined by shotgun cloning performed as described previously [1]. Sequence analysis of the DNA fragment surrounding the $bla_{\text{OXA-244}}$ gene revealed that it was part of a truncated Tn*1999.2* transposon, made of two copies of insertion sequences IS*1999* and a single IS*1R* element inserted into one of the IS*1999* copies [4]. Compared with the structure identified in pOXA-48a, an inverted orientation of the truncated Tn*1999.2* transposon was found in *E. coli* VAL (Fig. 1). Further analysis showed that the $bla_{\text{OXA-244}}$ gene was bracketed by two IS*1R* copies forming an IS*1R*-made composite transposon. This 21 852-bp transposon was inserted into a gene encoding an intrinsic endonuclease from *E. coli*, further supporting a chromosomal integration of this IS*1R*-made transposon. Identification of a 9-bp target site duplication (TGAATTGCT) at both extremities of the IS*1R*-made transposon was the signature of a transposition event. IS*1R*-made composite transposons harbouring the $bla_{\text{OXA-48}}$ gene and integrated into the chromosome of *E. coli* isolates from Lebanon have been recently described [5].

This study characterised OXA-244 possessing a weaker ability to hydrolyse imipenem and temocillin compared with OXA-48. Identification of such a variant raises again the issue of the threshold to be chosen for classifying a β-lactamase as a carbapenemase or not. Along with OXA-232, OXA-244 is another OXA-48 variant possessing a weaker ability to hydrolyse temocillin. Since this molecule has been integrated in screening culture media for detecting carbapenemase-producers, it might be interesting to evaluate the performances for detection of all those producers of OXA-48-like variants. ST38-type *E. coli* isolates harbouring a chromosomal $bla_{\text{OXA-48}}$ gene have been recovered from Lebanon, Egypt, Turkey, Switzerland and France [3]. Dissemination of OXA-48-like-producing *E. coli* isolates might therefore be linked to the dissemination of the ST38 clone at least in several countries [3,5]. Nevertheless, this diffusion has also been shown to be related to the mobility of $bla_{\text{OXA-48}}$-carrying IS*1R*-made composite transposons, inserted into different loci among various *E. coli* backgrounds [5].

The nucleotide sequence data reported in this work has been deposited in the GenBank nucleotide database under accession no. KR364794.

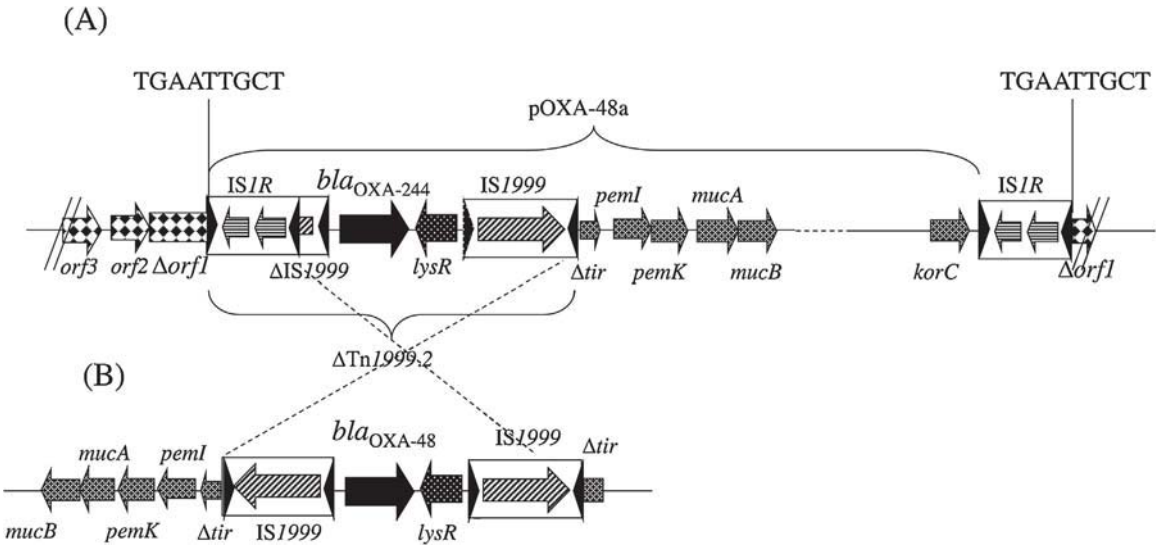


**Fig. 1.** Schematic map of (A) the transposon structure and surrounding sequences in *Escherichia coli* VAL and (B) the transposon Tn*1999* in reference plasmid pOXA-48a. Open reading frames (ORFs) are shown as arrows or as horizontal boxes with an arrow indicating the orientation of the coding sequence. Target site duplications (TGAATTGCT) are represented by black bars. *orf1*, *orf2* and *orf3* were similar to ORFs identified on *E. coli* chromosomes.


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## Competing interests

None declared.

## Ethical approval

Not required.

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