Escherichia coli carrying the *bla*_{CTX-M-15} gene of ST648

*bla*_{CTX-M} genes have emerged as the dominant genes encoding extended-spectrum β -lactamases in the world. Among more than 90 *bla*_{CTX-M} genes that have been identified so far, *bla*_{CTX-M-15} is the most widespread variant. Although *Escherichia coli* of O antigen type 25 (O25) and sequence type 131 (ST131) is largely responsible for the intercontinental spread of *bla*_{CTX-M-15}, some other sequence types have also been found associated with this gene. During a local surveillance of *bla*_{CTX-M} genes, an isolate of ST648 carrying *bla*_{CTX-M-15} was identified and is reported here. As an ST648 isolate carrying *bla*_{CTX-M-15} was found in the USA (Sidjabat *et al.*, 2009) recently, the findings here together with the USA report suggest a possible intercontinental spread of this lineage.

E. coli clinical isolate WCE227 was collected from a urine sample and was resistant to cefotaxime (MIC >32 μ g ml⁻¹) and ceftazidime (MIC >16 μ g ml⁻¹) as determined by the Phoenix automated microbiology system (BD). The WCE227 was hospital acquired as it was recovered from a patient hospitalized for more than 48 h, although the patient did not receive antimicrobial agents prior to sample collection. The *bla*_{CTX-M} gene was detected and was subsequently identified as *bla*_{CTX-M-15} by PCR and sequencing as described elsewhere (Zong *et al.*, 2008).

WCE227 was identified as being of the O25b subtype by O25b allele PCR. Phylogenetic group typing (Clermont *et al.*, 2000) revealed that WCE227 belonged to group D (subgroup D₁, having *chuA* but lacking *yjaA* and Tsp4.C2). WCE227 was of ST648 as determined by multilocus sequence typing (MLST) following an established scheme based on seven housekeeping genes (*adhA*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) available through the University College Cork MLST database (<http://mlst.ucc.ie/mlst/dbs/Ecoli>).

Since *bla*_{CTX-M-15} usually co-exists with a few other resistance determinants, *aac*(3)-II,

*bla*_{TEM}, *bla*_{OXA-1} (also called *bla*_{OXA-30}) and plasmid-encoded quinolone resistance determinants including *qnrA*, *qnrB*, *qnrS* and *qepA*, were screened by PCR, and *aac*(6')-Ib-cr was identified by sequencing. WCE227 had *bla*_{OXA-1} and *aac*(6')-Ib-cr but no others. WCE227 were resistant to trimethoprim (TMP)/sulfamethoxazole and had a class 1 integron with the *dfrA17*-*aadA5* cassette array that was determined by PCR and sequencing. Class 1 integrons contain *sulI*, a sulfonamide-resistance gene, in the 3' conserved segment. *dfrA17* encodes TMP-insensitive dihydrofolate reductases conferring resistance to TMP. As WCE227 was resistant to ciprofloxacin, the *gyrA* allele was partially sequenced. This revealed Ser83Tyr and Asp87Asn substitutions that have been seen in fluoroquinolone-resistant isolates before (Cagnacci *et al.*, 2008).

Mating was carried out in brain heart infusion broth (Oxoid) with *E. coli* DH5 α Rf, a spontaneous rifampicin-resistant mutant of DH5 α (Δ *lacZ*) as the recipient strain. WCE227 was sensitive to rifampicin and transconjugants were selected on 4 μ g cefotaxime ml⁻¹ plus 250 μ g rifampicin ml⁻¹. In WCE227, *bla*_{CTX-M-15} was carried by a conjugative plasmid, for which the incompatibility (Inc) group could not be assigned by PCR-based replicon typing (Carattoli *et al.*, 2005). Surprisingly, *aac*(6')-Ib-cr and *bla*_{OXA-1} were not co-transferred with *bla*_{CTX-M-15} in WCE227, in contrast to the findings of reports from elsewhere. The *dfrA17*-*aadA5* cassette array was also not located on the plasmid carrying *bla*_{CTX-M-15} in WCE227.

ST648 isolates carrying *bla*_{CTX-M} have been seen before, including two isolates carrying *bla*_{CTX-M-15} from the USA (Sidjabat *et al.*, 2009) and two carrying *bla*_{CTX-M-1} or *bla*_{CTX-M-32} from Spain (Blanco *et al.*, 2009). All of those ST648 isolates like WCE227 belonged to phylogenetic group D. Interestingly, ST648 without *bla*_{CTX-M-15} but carrying *aac*(6')-Ib-cr had been found in London, UK, prior to the

epidemic of *bla*_{CTX-M-15} (Jones *et al.*, 2008). The findings from the UK together with the fact that *aac*(6')-Ib-cr was not located on the plasmid carrying *bla*_{CTX-M-15} in WCE227 suggest that the ST648 lineage acquired the two genes independently. This is in contrast to the ST131 lineage, for which *bla*_{CTX-M-15} and *aac*(6')-Ib-cr were usually co-transferred on individual plasmids, most of which were FII-like.

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