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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 μg ml^{-1}) and ceftazidime (MIC >16 μg 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 D$_1$, having *chuA* but lacking *yjaA* and Tspe4.C2). WCE227 was of ST648 as determined by multilocus sequence typing (MLST) following an established scheme based on seven housekeeping genes (*adk*, *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 *sul1*, 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* DH5αRf, a spontaneous rifampicin-resistant mutant of DH5α (Δ*lacZ*) as the recipient strain. WCE227 was sensitive to rifampicin and transconjugants were selected on 4 μg cefotaxime ml^{-1} plus 250 μg 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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Zhiyong Zong and Rujia Yu

Center of Infectious Diseases, West China Hospital, Sichuan University, Chengdu, PR China

Correspondence: Zhiyong Zong (zongzhiyong@gmail.com)

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