Sequence type 38 *Escherichia coli* carrying *bla*_{CTX-M-14}

*bla*_{CTX-M} genes have emerged as the most common gene family 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-14} appears to be the second most widespread variant following *bla*_{CTX-M-15}, and is particularly common in East Asia, Canada and Spain. The intercontinental spread of *bla*_{CTX-M-15} is largely due to *Escherichia coli* of O antigen type 25 (O25) and sequence type 131 (ST131). Despite O25 ST131 *E. coli* having also been found to carry *bla*_{CTX-M-14} (Mora *et al.*, 2011; Suzuki *et al.*, 2009; Zong & Yu, 2011), some other STs have also been found associated with *bla*_{CTX-M-14}. During a local surveillance of *bla*_{CTX-M} genes, an isolate of ST38 carrying *bla*_{CTX-M-14} was identified and is reported here.

E. coli clinical isolate WCE329 was collected from a urine sample and was resistant to cefotaxime (MIC >32 $\mu\text{g ml}^{-1}$) as determined by the Phoenix automated microbiology system (Becton Dickinson). WCE329 had a community origin since it was recovered from a sample collected on admission and the host patient had no visits to medical facilities within the previous 3 months. The patient also had no foreign travel within the previous 1 year. The *bla*_{CTX-M} gene was detected, and was subsequently identified as *bla*_{CTX-M-14} by PCR and sequencing as described previously (Zong *et al.*, 2008).

WCE329 was identified as subtype O25b by O25b allele PCR (Clermont *et al.*, 2009). Phylogenetic group typing (Clermont *et al.*, 2000) revealed that WCE329 belonged to group D (subgroup D₂, having *chuA* and TspE4.C2 but lacking *yjaA*). WCE329 was of ST38 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 an *E. coli* MLST database (<http://mlst.ucc.ie/mlst/dbs/Ecoli>). ST38 and ST131 are distinct, without any identical alleles of the seven housekeeping genes. According to the MLST database, *E. coli* of ST38 are globally distributed, and have been found in Australia, Brazil, France, Germany, Nigeria and UK. However, only two ST38 isolates carrying *bla*_{CTX-M-14} were reported before, one from Japan (Suzuki *et al.*, 2009) and the other from Spain (Oteo *et al.*, 2009). The identification of a ST38 isolate carrying *bla*_{CTX-M-14} in China adds new evidence towards the geographical distribution of this ST.

Since *bla*_{CTX-M} 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*, *qepA* and *aac*(6')-Ib-cr were screened by PCR (Cattoir *et al.*, 2007; Yamane *et al.*, 2008; Zong *et al.*, 2008). WCE329 had *bla*_{TEM} only, which was identified as *bla*_{TEM-1b} by sequencing. WCE329 was

resistant to trimethoprim (TMP)/sulphamethoxazole and had a class 1 integron with the *dfrA12-gcuC-aadA2* cassette array detected by PCR and sequencing. Class 1 integrons contain *sul1*, a sulfonamide-resistance gene, in the 3'-conserved segment. *dfrA12* encodes a TMP-insensitive dihydrofolate-reductase conferring resistance to TMP.

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. WCE329 was sensitive to rifampicin, and transconjugants were selected on 4 μg cefotaxime ml^{-1} plus 250 μg rifampicin ml^{-1} . In WCE329, *bla*_{CTX-M-14} was carried by a conjugative plasmid (designated pWCE329 here) of the IncFII-related group determined as described elsewhere (Carattoli *et al.*, 2005; Osborn *et al.*, 2000), which did not contain *bla*_{TEM-1b} and the *dfrA12-gcuC-aadA2* cassette array. Sequencing revealed that pWCE329 harboured a variant of IncFII-related replicon RepFIIA identical to that of a plasmid from a local ST131 isolate (Zong & Yu, 2011) (GenBank accession no. GU462158), which was closest (94 % identity) to that of pSFO157 (Brunner *et al.*, 2006).

PCR mapping using the known structures containing *bla*_{CTX-M-14}, for example that in pKF3-70 (Yi *et al.*, 2010), as templates was employed to characterize the genetic

Fig. 1. Genetic context of *bla*_{CTX-M-14} in WCE329. *orf1* and *yadA*, function unknown; *yacC*, putatively encoding a polymerase III, epsilon subunit; *orf2*, putatively encoding a plasmid stabilization system protein; *yacA*, putatively encoding an ATPase component of ABC transporters. *repA4* and *repA1* belong to RepFIIA. The left-hand inverted repeat (IRR) and the alternative right-hand inverted repeat (IRR2) of *ISEcp1* are indicated by black vertical lines and the 5 bp DR, CATT A, are shown. IRR and IRR2 of *ISEcp1* had 9 out of 14 nt in common.

context of *bla*_{CTX-M-14} in WCE329. The *bla*_{CTX-M-14} was adjacent to a complete version of the insertion sequence *ISEcp1* upstream in WCE329 (Fig. 1). *ISEcp1* abutted a locus containing four genes, *etiA*, *B*, *C* and *D*, putatively encoding an iron ABC transporter that might be a virulence factor. The *stbB* gene, which was part of the plasmid backbone and specified the StbB protein involved in plasmid partitioning, was found upstream of the *eti* locus. A truncated version of *IS903* was located downstream of *bla*_{CTX-M-14} and abutted a *malB* (also referred as *lamB*) gene that encoded maltoporin, a lambda phage receptor protein, at the other end. Several genes were identified between *malB* and RepFIIA (Fig. 1). Direct target repeated sequences (DR), CATTA, were found abutting the left-hand inverted repeat of *ISEcp1* at one end and *malB* at the other. The presence of the characteristic 5 bp DR suggested that *ISEcp1* was able to recognize a sequence in *malB* as its alternative right-hand inverted repeat (IRR2; alignment between IRR and IRR2 shown in Fig. 1) and then mobilized the *bla*_{CTX-M-14}-*IS903Δ*-*malBA* structure. This genetic context is the same as those on pKF3-70 from *Klebsiella pneumoniae* in Zhejiang province (Yi *et al.*, 2010) and on pHK01 from *E. coli* in Hong Kong (GenBank accession no. HM355591), China. An almost identical context was also present on pEG356 from *Shigella sonnei* in Vietnam (GenBank accession no. FN594520) except that the *bla*_{CTX-M} gene was *bla*_{CTX-M-24}, a point mutant of *bla*_{CTX-M-14}. These findings suggested that the *ISEcp1*-*bla*_{CTX-M}-*IS903Δ*-*malBA* structure might have served as one of the common vehicles mediating the

spread of *bla*_{CTX-M-14} and its variants in East Asia.

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Chunhong Peng^{1,2} and Zhiyong Zong¹

¹Center of Infectious Diseases, West China Hospital, Sichuan University, Chengdu, PR China

²Department of Respiratory Medicine, West China Hospital, Sichuan University, Chengdu, PR China

Correspondence: Zhiyong Zong (zongzhiy@scu.edu.cn)

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