Emergence of pandemic B2-O25b-ST131 *Escherichia coli* harbouring *bla*_{CTX-M-3}, *bla*_{CTX-M-27} and *qnrS1* genes

Escherichia coli sequence type 131 (ST131) producing extended-spectrum β -lactamases (ESBLs) is emerging as a worldwide pandemic clone causing community and hospital-acquired infections (Rogers *et al.*, 2011). Among the ESBLs, CTX-M is the most prevalent in ST131, while TEM, SHV and CMY have been infrequently detected. Fluoroquinolone resistance is also common amongst ST131, and the aminoglycoside-modifying enzyme AAC(6')-Ib-cr, which also contributes to quinolone resistance, is frequently associated with ST131. However, the Qnr proteins mediating low-level quinolone resistance, such as QnrA, QnrB and QnrS, have been infrequently described in the ST131 clone (Pomba *et al.*, 2009). During a local surveillance of multidrug-resistant *E. coli* from humans and animals, an ST131 isolate carrying *bla*_{CTX-M-3}, *bla*_{CTX-M-27} and *qnrS1* genes was identified and is reported here.

E. coli clinical isolate GZ72w84 was isolated from a urine sample from an elderly woman at her annual personal physical examination at a hospital in Guangzhou (China). The volunteer had not been exposed to antibiotics or to a hospital environment in the 3 months prior to sample collection. The MICs were determined by standard broth microdilution assays, performed and interpreted according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2005). GZ72w84 was considered a multiresistant isolate, resistant to ampicillin, cephalosporins (ceftriaxone, cefotaxime, cefoxitin, ceftazidime), tetracycline, trimethoprim-sulfamethoxazole, streptomycin, chloramphenicol, nalidixic acid and fluoroquinolones (ciprofloxacin, norfloxacin and levofloxacin) and susceptible to imipenem, meropenem, gentamicin and amikacin.

GZ72w84 was identified as O25b subtype by O25b allele PCR (Clermont *et al.*, 2008). Phylogenetic group typing (Clermont *et al.*, 2000) revealed that GZ72w84 belonged to

group B2. GZ72w84 was of ST131, which was determined based on detecting a combination of two single nucleotide polymorphisms that are unique to ST131 within the *pabB* gene (Dhanji *et al.*, 2010).

The double-disc synergy test was used to test for ESBL production, then specific PCR amplification and sequencing was performed to determine the *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA} and *bla*_{CTX-M} genes (Kim *et al.*, 2005). Interestingly, two *bla*_{CTX-M} genes (*bla*_{CTX-M-3} and *bla*_{CTX-M-27}) were identified in GZ72w84.

As GZ72w84 was resistant to nalidixic acid and fluoroquinolones, quinolone resistance-determining regions of the *gyrA* and *parC* genes were amplified and sequenced (Yue *et al.*, 2008). GZ72w84 showed four amino acid substitutions: Ser83Lys and Asp87Asn in the *gyrA* gene and Ser80Ile and Glu84Val in the *parC* gene. The plasmid-borne quinolone resistance genes *qnrA*, *qnrB*, *qnrS* and *qepA* were also screened for by PCR as described previously (Yue *et al.*, 2008), but only *qnrS* was detected and subsequently identified as *qnrS1* by sequencing. GZ72w84 also carried the *aac*(6')-Ib gene but without the *cr* variant. For class 1 integron bearing *ISCR1* participating in the mobilization of *bla*_{CTX-M} genes, a class 1 integron with the *cmlA-aac*(6')-Ib cassette array was obtained by PCR and sequencing. The *qacEΔ1-sul1* genes also presented in the 3'-conserved region of this class 1 integron.

Mating was carried out using the filter method with C600, a streptomycin-resistant strain, as the recipient. Transconjugants were selected on MacConkey agar containing cefotaxime (2 mg l⁻¹) and streptomycin (1000 mg l⁻¹), but only transconjugants carrying *bla*_{CTX-M-3} were obtained. A ~54 kb plasmid was extracted from the transconjugants of GZ72w84 by alkaline lysis, and was then determined to be incompatibility group IncN by PCR-based

replicon typing (Carattoli *et al.*, 2005). The *qnrS1* gene could be co-transferred with *bla*_{CTX-M-3}, which was confirmed by Southern blot hybridization; in contrast, *bla*_{CTX-M-27} and the *cmlA-aac*(6')-Ib cassette array were not located on the plasmid carrying *bla*_{CTX-M-3} in GZ72w84. Nucleotide sequence analysis of the surrounding region of *bla*_{CTX-M-3} revealed *ISEcp1* 127 bp upstream and *orf477* downstream.

ST131 isolates carrying *bla*_{CTX-M-3} or *bla*_{CTX-M-27} have been described before (Rogers *et al.*, 2011). To our knowledge, this is the first study to describe the presence of CTX-M-3 and CTX-M-27 in one *E. coli* ST131 isolate. Interestingly, the ST131 clone producing CTX-M-3 and/or CTX-M-27 did not produce OXA-1 or TEM-1. The clone ST131 isolates producing CTX-M-3, isolated from the UK, were less multiresistant than those with the CTX-M-15 enzyme (Woodford *et al.*, 2009). Usually, clone ST131 strains producing CTX-M-15 carry *bla*_{TEM}, *bla*_{OXA-1}, *aac*(6')-Ib-cr, *catB4* and *tetA* resistance genes, but most of these genes are absent in clone ST131 strains producing CTX-M-3. However, GZ72w84 exhibited a multidrug-resistance phenotype, including resistance to expanded-spectrum cephalosporins, fluoroquinolones, trimethoprim-sulfamethoxazole, chloramphenicol and aminoglycosides, and harboured *qnrS1*, *int11*, *sul1*, *cmlA* and *aac*(6')-Ib genes, in addition to two *bla*_{CTX-M} genes. This is believed to be the first time that *qnrS1* has been found in clone ST131 and notably shown to co-transfer with CTX-M-3. The increasing occurrence of resistance genes in clone ST131 suggests that this lineage could acquire transferable resistance elements as independent events from an external source, and we should remain alert for this. More formal evaluations of the epidemiology and clinical impact of clone ST131 in China are required.

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