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# **Escherichia coli ST131 producing CTX-M-15 in Australia**

**Hanna E. Sidjabat, Petra Derrington, Graeme R. Nimmo, David L. Paterson**

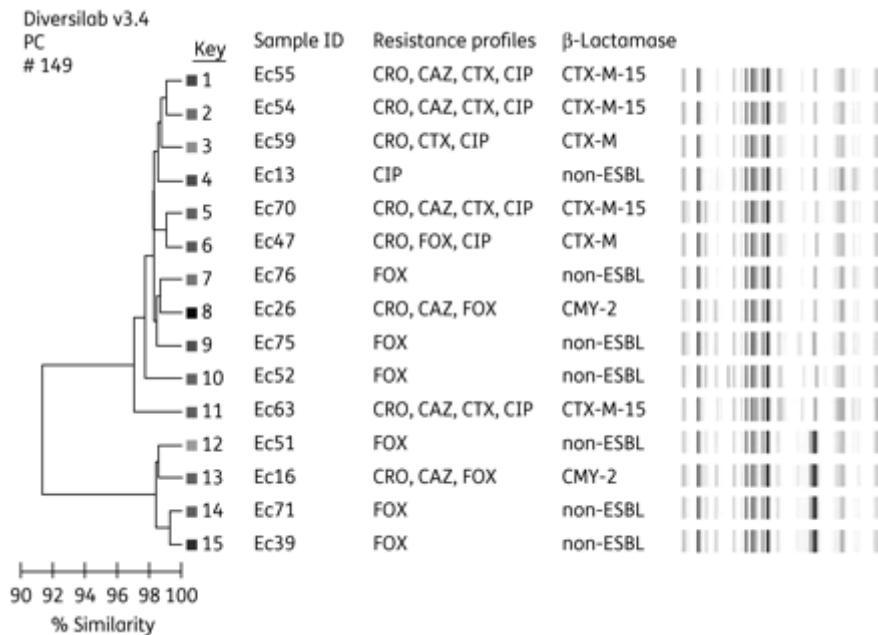
*Sir,*

*Escherichia coli* clonal group ST131 has been reported from many parts of the world and is frequently associated with production of CTX-M-type extended-spectrum  $\beta$ -lactamase (ESBL), in particular CTX-M-15.<sup>1,2</sup> Clonal group ST131 may be associated with other  $\beta$ -lactamases or may be resistant to fluoroquinolones; some isolates may be cephalosporin susceptible.<sup>3</sup> Thus far, there are no reports of the clonal group ST131 *E. coli* in Australia. In this study, 49 *E. coli* clinical isolates resistant to cephalosporins and/or fluoroquinolones collected from six clinical microbiology laboratories in south-east Queensland between 2008 and 2009 were investigated for the presence of this clonal group.

All isolates were confirmed by disc diffusion using the CLSI guidelines as resistant to at least one of the following antibiotics: ciprofloxacin; ceftazidime; or ceftaxime.<sup>4</sup> The phylogenetic groups (A, B1, B2 or D) of all isolates were determined by an established multiplex PCR-based method.<sup>5</sup> Further, all isolates were screened to determine if they belonged to the O25b-ST131 clone by using a PCR-based method.<sup>6</sup> Sequence types (STs) were confirmed by multilocus sequence typing (MLST) for isolates that were positive for O25b-ST131 (<http://mlst.ucc.ie/mlst/dbs/Ecoli>). Mechanisms of resistance to  $\beta$ -lactams due to ESBLs or AmpC-type  $\beta$ -lactamases were sought phenotypically and genotypically in these isolates according to previously published methods.<sup>7</sup>

Additionally, isolates underwent repetitive sequence-based PCR (rep-PCR) using the DiversiLab® System (bioMérieux, Melbourne, Australia) to determine their clonal relatedness. The DNA from the 49 isolates was extracted using an Ultraclean Microbial DNA Isolation Kit (MO BIO Laboratories, Inc., CA, USA) and amplified using the DiversiLab Escherichia Kit for DNA fingerprinting (bioMérieux) following the manufacturer's instructions. Briefly, the mastermix included 2 µL of genomic DNA (25–50 ng/µL), 2.5 U of AmpliTaq polymerase (Applied Biosystems, NJ, USA), 2 µL of kit-supplied primer mix and 2.5 µL of 10× PCR buffer (Applied Biosystems) for each reaction. The PCR conditions were as follows: initial denaturation at 94°C for 2 min; followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 70°C for 90 s; and a final extension at 70°C for 3 min. Separation and detection of rep-PCR products was performed using microfluidic LabChip electrophoresis (DiversiLab System, bioMérieux) and analysis was performed with DiversiLab software version v.3.4.38. The Pearson correlation coefficient was used to analyse and calculate genetic similarity coefficients among all samples. The unweighted pair-group method of averages (UPGMA) was employed to automatically compare the rep-PCR profiles and create corresponding dendrograms. Percentage similarity for *E. coli* was set at  $\geq 95\%$ .

A total of 15 *E. coli* isolates were determined as belonging to clonal group O25b-ST131; of these, six produced CTX-M-type ESBLs (four isolates produced CTX-M-15) and two produced CMY-2. All except three isolates originated from urine. The isolates analysed by the DiversiLab System showed that the ST131 *E. coli* clone consisted of two related DiversiLab profiles with ~92% similarity. Within each profile there was >95% similarity (Figure 1). This ST131 *E. coli* clone showed a rep-PCR pattern very distinct from that of other *E. coli* commonly found in our laboratory. Our O25b-ST131 *E. coli* DiversiLab profiles were similar to published profiles from the UK.<sup>8</sup>



Dendrogram of rep-PCR showing similarity among 15 *Escherichia coli* isolates with a gel image of rep-PCR patterns. Resistance profile represents antibiotics to which the isolate was resistant. Non-ESBL indicates negative phenotypic tests for ESBL production and the absence of genes encoding SHV- or CTX-M-type ESBLs. CRO, ceftriaxone; CAZ, ceftazidime; CTX, cefotaxime; CIP, ciprofloxacin; FOX, ceftioxitin.

We have not been able to ascertain the travel history of the patients, but believe it unlikely that all affected patients have travelled overseas to countries with established endemic ST131 *E. coli*. The age range of patients affected by this clonal group was from 1-day-old babies to nursing home residents in their 80s. This short report extends the documented geographical spread of CTX-M-15-producing ST131 *E. coli* to Australia. More formal evaluations of the epidemiology and clinical impact of this clone in Australia are underway.

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