References


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Escherichia coli ST131 producing CTX-M-15 in Australia

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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 β-lactamases (ESBL), in particular CTX-M-15.1,2 Clonal group ST131 may be associated with other β-lactamases or may be resistant to fluoroquinolones; some isolates may be cephalosporin susceptible.1 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; cefotaxin; ceftazidime; or cefotaxime.4 The phylogenetic groups (A, B1, B2 or D) of all isolates were determined by an established multiplex PCR-based method.5 Further, all isolates were screened to determine if they belonged to the O25b-ST131 clone by using a PCR-based method.6 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 β-lactams due to ESBLs or AmpC-type β-lactamases were sought phenotypically and genotypically in these isolates according to previously published methods.7

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 Ultrapure 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 master-mix 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 10x 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 ≥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.8

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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Identification of a bla_vim-4 gene in the internationally successful Klebsiella pneumoniae ST11 clone and in a Klebsiella oxytoca strain in Hungary

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Sir,

The VIM-type metallo-β-lactamases (MBLs) have been detected worldwide in Pseudomonas aeruginosa and Acinetobacter spp., and, more recently, in many species of Enterobacteriaceae. After the first detection of VIM-4 in P. aeruginosa in Greece in 2001,1 these strains were isolated in many countries, including Hungary, where VIM-4-producing P. aeruginosa and Aeromonas hydrophila were reported.2,3

In Hungarian clinical microbiological laboratories, the screening for presumptive carbapenemase producers is performed according to Hungarian guidelines, which are based on the CLSI recommendations.4 The putative production of MBLs is tested by the modified Hodge test, and disc tests containing imipenem and/or ceftazidime either alone or combined with EDTA. In 2009, 5% of Klebsiella spp. (1.4% of Enterobacteriaceae) isolates from the BP1 centre and 4.76% of Klebsiella spp. (2.4% of Enterobacteriaceae) isolates from the BP2 centre were carbapenem-resistant non-carbapenemase producers. No imipenem or meropenem non-susceptible strains were isolated during this year. Based on screening and confirmatory tests, one carbapenem-susceptible Klebsiella pneumoniae strain (KP3686) isolated from bronchoalveolar lavage (BP1 centre, February 2009) and one carbapenem-susceptible Klebsiella oxytoca strain (KOS294/9) isolated from stool (BP2 centre, July 2009) proved to be MBL producers. The aim of our study was to characterize the first Hungarian MBL-producing K. pneumoniae KP3686 and K. oxytoca KOS294/9 isolates.

MICs of antimicrobial agents were determined by Etest (bio-Mérieux, Marcy l’Etoile, France) (Table 1). In the PCR assays, primers targeting the 5’ conserved sequence (CS) and the 3’ CS of class 1 integrons,2,3 and primers specific to bla_tem, bla_shv, blaCTX-M-5 and bla_vim were used. The nucleotide sequences were determined using an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

In KP3686, the chromosomally encoded non-extended-spectrum β-lactamase (non-ESBL)-type bla_shv11, plasmid-carried blaCTX-M-15 and bla_tem, and the class 1 integron-located bla_vim-1 genes were detected (Table 1). In KOS294/9, only the bla_vim-1-carrying class 1 integron was found. Both integrons carried two resistance gene cassettes, namely an aac(6’)-Ib (so-called aac44) gene in the first position, followed by a bla_vim-1 gene cassette. The results of integron sequencing showed the same VIM-4-containing class 1 integron in both isolates. Furthermore, the integron was found to be identical to that previously characterized from P. aeruginosa strains that originated from southern Hungary and from the first MBL-producing A. hydrophila strain.2,3 Their nucleotide sequences were assigned to GenBank under the accession numbers GU181265 for KP3686 and GU181269 for KOS294/9.

References

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