

## Research letters

MIC increases. Positions 965–967 correspond to the triple-base-pair mutation involved in the high-level tetracycline resistance in *H. pylori*,<sup>2</sup> whereas nucleotide 1054 contacts the A-site tRNA.<sup>6</sup> In contrast, the G346A mutation is located in a 16S rRNA region not closely associated with tetracycline binding.

For *M. pneumoniae*, two mutations were found in the broth-selected mutants and were associated with 2- to 16-fold increases in MICs of the three tetracyclines studied, in comparison with those of the parental strain FH. The two mutations described for this species, G1193A and T968C, are located very close to or contact the primary tetracycline binding site, respectively.

In summary, this is the first description of mutations in 16S rRNA associated with decreased susceptibility to tetracyclines in human mycoplasmas. However, what real effect these new mutations have on the tetracycline susceptibility of both mycoplasmas has yet to be determined.

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### Transparency declarations

None to declare.

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### Multidrug-resistant *Providencia stuartii* expressing extended-spectrum $\beta$ -lactamase PER-1, originating in Kosovo

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Sir,

The current emergence and dissemination of clavulanic acid-inhibited, extended-spectrum  $\beta$ -lactamase (ESBL)-producing Enterobacteriaceae represent a global threat, as they are difficult to trace and eradicate, and cause both nosocomial and community-acquired infections.<sup>1</sup> Although the unexplained worldwide escalation of CTX-M-type enzymes is of major concern, other types of ESBLs may be prevalent in more restricted geographical areas. The ESBL PER-1 has been identified in several Gram-negative species including *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.<sup>2</sup> This ESBL determinant has been identified in several countries such as France, Italy, Poland and South Korea and is particularly widespread in Turkey.<sup>2</sup>

We report here a multidrug-resistant *Providencia stuartii* isolate, which was recovered in 2004 from a 60-year-old patient who had a femur head prosthesis implantation at the University Hospital Pristina, Kosovo. He subsequently developed a chronic infection, fistulations occurred and he was transferred to the University Medical Clinic of Orthopaedic Surgery, Liestal, Switzerland, where his femur head was removed. Cultures made from intra-operative biopsies yielded oxacillin-susceptible *Staphylococcus aureus*, *Enterococcus faecium* and *P. stuartii* isolates; the latter isolate was identified with the API32GN system (bioMérieux, Marcy l'Étoile, France). Disc diffusion and broth microdilution methods were used to determine its antibiotic susceptibility and results were interpreted according to CLSI criteria. *P. stuartii* isolate 166 was resistant to multiple antibiotics including oxyimino-cephalosporins, fluoroquinolones, chloramphenicol, tetracycline, trimethoprim, aminoglycosides (except amikacin) and colistin, but it remained susceptible to cephamycins and carbapenems. Synergy tests performed with discs containing ticarcillin/clavulanic acid and either ceftazidime or ceftipime indicated production of an ESBL. PCR with primers specific for known ESBL genes<sup>2</sup> identified *bla*<sub>PER-1</sub>, and PCR mapping showed this to be part of a Tn1213 composite transposon.<sup>3</sup> Plasmid-mediated Qnr-type determinants have been associated with ESBL genes, but isolate 166 lacked known Qnr-encoding genes.<sup>4</sup> Conjugation studies, with ceftazidime selection and *Escherichia coli* J53 (azide-resistant) as the recipient, showed that the *bla*<sub>PER-1</sub> gene was located on a 150 kb plasmid, which also conferred resistance to chloramphenicol, trimethoprim, aminoglycosides (except amikacin) and sulphonamides.

The patient was treated for 6 weeks in Switzerland with imipenem and vancomycin and recovered, but further follow-up was not possible because the patient returned to Kosovo.

There are no epidemiological data regarding ESBLs available for Kosovo. A survey recently performed in Bosnia and

Herzegovina identified SHV-5 producers among ESBL-positive enterobacterial isolates.<sup>5</sup> PER-1 ESBL has been identified in *A. baumannii* and *P. aeruginosa* in Turkey and Romania, and in *P. stuartii* in Italy.<sup>6</sup> This report of PER-1 in an enterobacterial isolate from Kosovo may indicate that the *bla*<sub>PER-1</sub> gene has spread in South Eastern Europe.

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## Transparency declarations

None to declare.

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## High prevalence of CTX-M-15-producing *Klebsiella pneumoniae* among inpatients and outpatients with urinary tract infection in Southern India

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Sir,

The population of India of over one billion represents a potentially vast reservoir of antimicrobial resistance genes including those encoding extended-spectrum  $\beta$ -lactamases (ESBLs). Occurrence of ESBL-producing Enterobacteriaceae has been described in India mostly using phenotypic detection methods alone. The present study was conducted to identify the occurrence of ESBL genes in urinary isolates of *Klebsiella pneumoniae* collected in Southern India.

The study was conducted at two hospitals, one in Gulbarga and the other in Raichur, between July 2005 and March 2006. The two cities are ~150 km away from each other and have populations of ~440 000 and 160 000, respectively. A total of 1288 non-duplicate urine specimens were collected from 642 inpatients and 646 outpatients. *K. pneumoniae* was identified by standard biochemical tests including citrate, urease, indole, Voges–Proskauer, glucose and inositol. As a result, *K. pneumoniae* was recovered from 270 of 1288 urinary specimens. Among them, 115 (43%) were from inpatients and 155 (57%) were from outpatients. ESBL production was confirmed in 260 (96%) isolates using the disc method defined by the CLSI (2007). All these isolates produced  $\geq 5$  mm increase in zone diameters with either cefotaxime or ceftazidime discs when clavulanic acid was added. They represented 95% and 97% of the inpatient and outpatient isolates, respectively. Of those, 255 isolates were resistant to cefotaxime and were assigned to phenotypic Group I, which was further classified into subgroups depending on susceptibility to ceftazidime and cefepime (Table 1). Another five isolates with resistance to ceftazidime but not to cefotaxime were assigned to phenotypic Group II. The remaining 10 non-ESBL-producing isolates susceptible to both cefotaxime and ceftazidime were assigned to phenotypic Group III. For non- $\beta$ -lactam antimicrobials, 92% and 95% of the inpatient and outpatient isolates were resistant to ciprofloxacin, respectively. High rates of resistance to gentamicin (79% and 83% in inpatient and outpatient isolates, respectively) and amikacin (66% and 69% in inpatient and outpatient isolates, respectively) were also observed. Resistance to chloramphenicol, which is still commonly prescribed for infections caused by *K. pneumoniae* in India, was observed in 90% and 92% of the inpatient and outpatient isolates, respectively. All the study isolates were susceptible to ertapenem, except for one isolate which was intermediately resistant. No resistance to imipenem was identified.

A total of 35 isolates from all phenotypic groups and subgroups were subjected to PFGE analysis using restriction enzyme *Xba*I (New England Biolabs, Ipswich, MA, USA) and CHEF III DR electrophoresis system (Bio-Rad, Hercules, CA, USA). Forty-five percent of the isolates in the phenotypic Group I that were examined by PFGE belonged to pulsotype A (Table 1). This pulsotype was commonly observed regardless of the source hospitals or the patient locations. The isolates in the phenotypic Group II belonged to pulsotype D. A total of 60 isolates from various pulsotypes were then subjected to PCR analysis for the detection of *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub>, as described previously.<sup>1,2</sup> All the isolates belonging to the