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

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Transparency declarations

None to declare.

References

1. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. *Clin Microbiol Rev* 2005; **18**: 657–86.

2. Naas T, Poirel L, Nordmann P. Minor extended-spectrum β-lactamases. *Clin Microbiol Infect* 2008; **14** Suppl 1: 42–52.

3. Poirel L, Cabanne L, Vahaboglü H *et al.* Genetic environment and expression of the extended-spectrum β-lactamase bla_{PER-1} gene in Gram-negative bacteria. *Antimicrob Agents Chemother* 2005; **49**: 1708–13.

4. Cattoir V, Poirel L, Rotimi V *et al.* Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing enterobacterial isolates. *J Antimicrob Chemother* 2007; **60**: 394–7.

5. Uzunovic-Kamberovic S, Bedenic B, Vranes J. Predominance of SHV-5 β-lactamase in enteric bacteria causing community-acquired urinary tract infections in Bosnia and Herzegovina. *Clin Microbiol Infect* 2007; **13**: 820–3.

6. Perilli M, De Santis F, Mugnaioli C *et al.* Spread of Enterobacteriaceae carrying the PER-1 extended-spectrum β-lactamase gene as a chromosomal insert: a report from Italy. *J Antimicrob Chemother* 2007; **59**: 323–4.

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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 β-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-β-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_{TEM}, bla_{SHV} and bla_{CTX-M}, as described previously.[1,2] All the isolates belonging to the

Table 1. Phenotypic and genotypic characteristics of the *K. pneumoniae* clinical isolates

Phenotypic			Antibiogram											
Group	subgroup	No. of isolates ($n = 270$)	CAZ	CTX	FEP	FOX	ETP	IPM	CIP	CHL	GEN	AMK	ESBLs	Pulsotypes
I	a	155	R	R	R	S	S	S	R	R	S/I/R	S/I/R	CTX-M-15	A
	b	71	R	R	I	S	S	S	R	R	S/I/R	S/I/R	CTX-M-15	A
	c	18	I	R	S	S	S	S	R	S/I/R	S/R	S/I/R	CTX-M-15	B
	d	7	S	R	S	S/R	S	S	I/R	I/R	R/S	S	CTX-M-15	C
	e	4	R	R	S	S	S	S	R	R	R/S	R/S	CTX-M-15	A, B, K, L
II	—	5	R	S	S	S	S	S	S	I/S	S	S	SHV-12	D
III	—	10	S	S	S	S	S	S	S	I/R/S	S	S	none	miscellaneous

CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; FOX, cefoxitin; ETP, ertapenem; IPM, imipenem; CIP, ciprofloxacin; CHL, chloramphenicol; GEN, gentamicin; AMK, amikacin.
Susceptibility results were interpreted according to the CLSI criteria: R, resistant; I, intermediate; S, susceptible.

phenotypic Group I yielded amplicons consistent with $bla_{\text{CTX-M}}$. The gene was confirmed as $bla_{\text{CTX-M-15}}$ in representative isolates by sequencing the entire open-reading frame using the following external primers: CTX-M-15-SF, 5′-CACACGTGGAATTTA GGGACT-3′ and CTX-M-15-SR, 5′-GCCGTCTAAGGCGAT AAACA-3′. These results were consistent with the findings in a previous study reporting the predominance of $bla_{\text{CTX-M-15}}$ in a small number of isolates from Southern India.[3] Some of the Group I isolates were positive for bla_{TEM} or bla_{SHV}. The sequences of representative amplicons were consistent with $bla_{\text{TEM-1}}$ and either $bla_{\text{SHV-1}}$ or $bla_{\text{SHV-28}}$, respectively, all of which are non-ESBL genes. All five isolates in Group II were positive for $bla_{\text{SHV-12}}$, an ESBL gene, and negative for bla_{TEM} or $bla_{\text{CTX-M}}$.

The prevalence of ESBL producers in this study was strikingly high, particularly given the fact that more than half of the isolates were obtained from outpatients. One of the reasons contributing to the high prevalence of ESBL may be the crowded hospital conditions precluding implementation of optimal hygiene practices. The epidemic in the community is then likely fuelled by unrestricted use of antimicrobials that may be purchased without prescription.[4] A recent study reported that ESBL-producing Enterobacteriaceae, including *K. pneumoniae*, were responsible for community-onset infections in India.[5] Dissemination of ESBL-producing *K. pneumoniae* in the community has a serious implication in the empirical management of complicated urinary tract infections caused by this organism, given their tendency for co-resistance to non-β-lactam classes of antimicrobials, as was observed in the present study. CTX-M-15 is known to be an ESBL that has peculiar association with community-onset *Escherichia coli* infections.[6] It may therefore be speculated that CTX-M-15-producing *E. coli* is already established in the community in this area and that the responsible gene is being exchanged between *E. coli* and *K. pneumoniae* by means of conjugal transfer, both in the healthcare and in the community environments.

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Transparency declarations

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References

1. Kruger T, Szabo D, Keddy KH *et al.* Infections with nontyphoidal *Salmonella* species producing TEM-63 or a novel TEM enzyme, TEM-131, in South Africa. *Antimicrob Agents Chemother* 2004; **48**: 4263–70.

2. Edelstein M, Pimkin M, Palagin I *et al.* Prevalence and molecular epidemiology of CTX-M extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals. *Antimicrob Agents Chemother* 2003; **47**: 3724–32.

3. Ensor VM, Shahid M, Evans JT *et al.* Occurrence, prevalence and genetic environment of CTX-M β-lactamases in Enterobacteriaceae from Indian hospitals. *J Antimicrob Chemother* 2006; **58**: 1260–3.

4. Ray K, Mukhopadhyay S, Dutt D *et al.* Cross-sectional study of consumption, compliance and awareness about antibiotic utilisation amongst the urban community in Kolkata. *J Indian Med Assoc* 2003; **101**: 7, 9–10.

5. Gupta V, Datta P. Extended-spectrum β-lactamases (ESBL) in community isolates from North India: frequency and predisposing factors. *Int J Infect Dis* 2007; **11**: 88–9.

6. Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V *et al.* Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. *J Antimicrob Chemother* 2008; **61**: 273–81.

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Daptomycin resistance in *Enterococcus faecalis* prosthetic valve endocarditis

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