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RESEARCH NOTE

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Predominance of SHV-5 β-lactamase in enteric bacteria causing community-acquired urinary tract infections in Bosnia and Herzegovina

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ABSTRACT

The β-lactamases of 14 non-duplicate *Klebsiella pneumoniae* isolates and five *Escherichia coli* isolates from urine samples obtained from outpatients were characterised by isoelectric focusing, substrate profile determination, PCR and sequencing of bla_{SHV} genes. Three *E. coli* K12 transconjugants were identified as isolates that produced SHV-5 β-lactamase. This report is the first description of SHV-5 β-lactamase among community isolates. Since the isolates showed distinct pulsed-field gel electrophoresis patterns, it was concluded that there was no clonal spread of bla_{TEM} and bla_{SHV} genes, and that dissemination of bla_{TEM} and bla_{SHV} genes was the result of exchange of plasmids among different clones.

Keywords Cefazidime, extended-spectrum β-lactamases, *Escherichia coli*, *Klebsiella pneumoniae*, resistance, SHV-5

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Plasmid-encoded resistance to broad-spectrum cephalosporins and aztreonam is becoming a widespread problem in clinical medicine [1]. An increase in the prevalence of extended-spectrum β-lactamases (ESBLs) causing community-acquired urinary tract infections has been observed among children aged 0–6 years in Zenica-Doboj Canton, Bosnia and Herzegovina [2,3]. The aim of the present study was to characterise the ESBLs found in *Klebsiella pneumoniae* and *Escherichia coli* isolates from urinary tract infections in this region.

The Laboratory for Sanitary and Clinical Microbiology, Cantonal Public Health Institution, Zenica serves a population of 331 229 in the Zenica-Doboj Canton of Bosnia-Herzegovina (112 471 males and 218 758 females). Between May 2004 and April 2005, 2059 enterobacterial isolates were obtained consecutively from single outpatient urine samples. Basic patient demographic data were recorded routinely for all urine samples. Antibiotic susceptibility to 15 antimicrobial agents was tested initially by disk diffusion according to CLSI recommendations [4], with the production of ESBLs being confirmed by double-disk synergy tests [5]. Nineteen ESBL-producing isolates were available for further testing. MICs of a wide range of antibiotics were determined by a two-fold microdilution test according to CLSI recommendations [4].

Transfer of resistance to oxymino cephalosporins was tested by conjugation (broth mating method), with *Esch. coli* strain A15R$^-$ (resistant to rifampicin) as the recipient [6]. Crude bacterial sonicates were subjected to isoelectric focusing on polyacrylamide gels with a pH range of 3.5–10 [7]. β-Lactamases were detected by staining the gels with nitrocefin (Oxoid, Basingstoke, UK). Reference strains producing TEM-1, TEM-2, SHV-1, SHV-2, SHV-3, SHV-4, SHV-5 and CTX-M-15 were used as pI standards.

Specific bla_{ESBL} genes were detected by PCR with primers specific for TEM, SHV and CTX-M β-lactamases (Table 1). PCR conditions comprised 94°C for 5 min, followed by 35 cycles of 95°C for 1 min, 55°C (68°C for SHV [8]) for 1 min and 72°C for 1 min. Reference strains producing TEM-1, TEM-2, SHV-1, SHV-2, SHV-3, SHV-4 and CTX-M-15 were used as positive controls [8]. The bla_{SHV} genes of the three transconjugants derived from the *K. pneumoniae* isolates 14213, 35117 and 35123 (see below) were sequenced using an ABI PRISM 377 Genetic Analyser (Applied Biosystems, Warrington, UK). Using the primer pairs listed above, all amplicons of the bla_{SHV} genes spanned the entire open reading frame.

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Table 1. Characterisation of β-lactamases and MICs for *Klebsiella pneumoniae* and *Escherichia coli* isolates producing extended-spectrum β-lactamases

Isolate	Age (years)	M/F	PCR-CTX-M[a]	PCR-TEM[b]	pI of enzyme	Frequency of conjugation	Co-transferred resistance markers	PFGE type	Antibiotic												
									AMX	AMC	CAZ	CAZ/CL	CTX	CRO	FEP	AMT	IMI	MEM	GM	NM	TM
K. pneumoniae																					
14213	56	F	–	–	82	10^{-3}	GM, NM, TM, T	1	≥1024	64	≥1024	1	32	64	32	≥1024	0.12	0.06	32	32	64
35117	0	M	–	–	82	10^{-3}	GM, NM, TM, T	2	≥1024	16	128	0.25	8	16	32	256	0.06	0.06	16	32	128
35123	64	M	–	–	82	10^{-5}	T, C	3	≥1024	32	256	0.5	32	32	16	512	0.12	0.03	16	32	128
37464	01	F	–	–	82	10^{-6}	T, C	4	≥1024	32	128	0.12	8	8	32	512	0.06	0.03	64	16	64
110	48	M	–	–	82	10^{-4}	T, C	5	≥1024	8	64	0.12	32	64	8	128	0.03	≤0.016	16	8	32
836	86	M	–	–	82	–		6	≥1024	16	128	0.25	16	64	8	128	0.06	≤0.016	64	16	32
3069	69	M	–	–	82	10^{-5}	None	7	≥1024	64	≥1024	1	64	128	16	≥1024	0.25	0.06	128	32	64
3075	74	F	–	–	8.2	10^{-4}	GM, NM, TM, C	8	≥1024	8	32	0.06	16	32	16	128	0.03	≤0.016	16	4	16
4897	70	M	–	–	8.2	–		9	≥1024	≥128	>1024	2	32	64	64	>1024	0.12	≤0.016	0.25	0.06	0.5
8329	0	F	–	–	8.2	10^{-6}	GM, NM, TM, T, C	10	≥1024	2	64	0.06	64	16	32	256	0.06	≤0.016	4	1	8
16454	01	M	–	–	8.2	–		11	≥1024	32	512	0.25	8	16	32	≥1024	0.25	0.06	16	8	32
15822	01	M	–	–	8.2	10^{-6}	GM, NM, TM, T, C	12	≥1024	2	16	0.06	8	8	4	32	0.5	0.25	16	16	128
17178	0	M	–	–	8.2	–		13	≥1024	≥128	≥1024	1	16	16	32	512	0.12	0.06	16	16	32
13086	0	M	–	–	8.2	10^{-3}	GM, NM, TM, T	14	≥1024	8	128	0.12	8	16	8	128	0.25	0.06	32	8	16
E. coli																					
32248	01	M	–	+	5.4	10^{-5}	None	1	≥1024	4	32	0.25	4	4	4	64	≤0.016	≤0.016	8	16	32
35955	72	M	–	+	5.4	10^{-3}	GM, NM, AK, C	2	≥1024	16	128	0.5	16	32	16	512	0.06	≤0.016	32	32	64
16	73	M	–	+	5.4	–		3	≥1024	4	32	0.12	8	16	8	32	≤0.016	≤0.016	64	32	128
83	68	M	–	+	5.4	–		4	≥1024	8	128	0.25	32	64	16	256	≤0.016	≤0.016	128	32	64
15911	01	M	–	+	5.4	10^{-4}	GM, NM, AK, C	5	≥1024	16	64	0.12	2	16	4	128	0.12	0.06	16	8	16

M/F, male/female; PFGE, pulsed-field gel electrophoresis; AMX, amoxycillin; AMC, amoxycillin–clavulanate; CAZ, ceftazidime; CAZ/CL, ceftazidime–clavulanate; CTX, cefotaxime; CRO, ceftriaxone; FEP, cefepime; AMT, aztreonam; IMI, imipenem; MEM, meropenem; GM, gentamicin; NM, netilmycin, AK, amikacin; TM, tobramycin; C, chloramphenicol; T, tetracycline.

[a]Sequences of primers: 5′-SCSATGTGCAGYACCAGTAA (MA1) and 5′-CCGCRATATGRTTGGTGGTG (MA-2).

[b]Sequences of primers: 5′-CGCCGGGTTATTCTTATTTGTCGC (OT3-A) and 5′-TCTTTCCGATGCCGCCGCCAGTCA (OT4-B).

Transconjugant plasmid DNA was extracted by alkaline lysis [9], digested with *Eco*RI and analysed by electrophoresis on agarose 0.8% w/v gels. Pulsed-field gel electrophoresis of *Xba*I-digested genomic DNA was performed using a CHEF-DRII system (Bio-Rad, Hemel Hempstead, UK) [10]. The images were processed using GelCompar software (Applied Maths, Sint-Martens-Latem, Belgium), and a dendogram was computed after band intensity correlation using global alignment with 2% optimisation and the unweighted pair-group method using arithmetical averages (UPGMA) clustering.

The 2059 enteric bacterial isolates included in the study comprised 68.1% *Esch. coli*, 23.7% *Klebsiella* spp., 6.8% *Proteus* spp., 0.7% *Citrobacter* spp. and 0.7% *Enterobacter* spp. ESBLs were detected by double-disk synergy tests and an eight-fold reduction in the ceftazidime MIC in the presence of clavulanic acid (2 mg/L) in 55 (2.7%) isolates, of which 44 were *Klebsiella* spp., eight were *Esch. coli*, and three were *Enterobacter* spp. ESBL producers were isolated from 42.6% of children aged ≤6 years. The prevalence of ESBL producers from males was double that of isolates from females (69.1% and 30.9%, respectively), resulting in incidences of 8.7% and 1%, respectively.

The antibiotic susceptibilities of 19 selected isolates are shown in Table 1. The addition of clavulanic acid to ceftazidime reduced the MIC to <2 mg/L. Imipenem and meropenem remained active (MICs <0.5 and <0.25 mg/L, respectively). Transfer of antibiotic resistance was achieved for ten *K. pneumoniae* and three *Esch. coli* isolates (Table 1). Resistance to non-β-lactam antibiotics was co-transferred with cephalosporin resistance in most cases.

Esch. coli isolates and their resulting transconjugants showed β-lactamase activity at a pI of 5.4, corresponding to TEM-1, while *K. pneumoniae* isolates and their resulting *Esch. coli* transconjugants showed β-lactamase activity at a pI of 8.2, corresponding to SHV-5. PCR detected bla_{SHV} genes in all *K. pneumoniae* strains and their transconjugants, and bla_{TEM} genes in all *Esch. coli* isolates. No CTX-M β-lactamase producers were found.

Sequencing of bla_{SHV} from three transconjugants (14213, 35117 and 35123) revealed two mutations: at Ambler amino-acid position 238 (GGC → AGC, glycine → serine), typical for all SHV ESBLs, and at Ambler amino-acid position 240 (GAG → AAG, glutamic acid → lysine), typical for SHV-5. Based on sequencing data, enzymes from all three tranconjugants were identified as SHV-5. Plasmid analysis after digestion with *Eco*R1 showed that the transconjugants harboured plasmids with distinct fingerprinting

patterns. The *K. pneumoniae* and *Esch. coli* isolates also showed distinct pulsed-field gel electrophoresis patterns (Fig. 1).

The overall prevalence of ESBL-producing isolates in the community (2.7%) found in this study corresponds to the prevalence found in hospital settings and private healthcare centres in other reports [11]. The present study revealed a higher prevalence of ESBL-producing isolates among *K. pneumoniae* than among *Esch. coli*, which is in contrast with previous studies, in which *Esch. coli* was the most prevalent ESBL-producing species isolated from outpatient samples [12]. This was probably a result of the large proportion of *Klebsiella* spp. isolated from community-acquired urinary tract infections, as *K. pneumoniae* is usually associated with hospital settings and complicated community-acquired urinary tract infections [12,13]. The median age of the patients (35 years) was lower than that in previous studies, and the finding that male patients were infected more frequently with ESBL-producing strains than female patients is also in contrast to previous reports [14,15].

Overall, the results indicate that microorganisms carrying ESBL genes are present in patients in the community in Bosnia and Herzagovina, as well as in other countries [13,14,16]. Transmission of ESBL producers from the hospital to the community cannot be excluded, particularly since there are reports of prolonged carriage of such strains after hospitalisation [17]. Furthermore, evidence of ESBL producers in cattle, poultry, dogs and cats suggests that food-producing animals and house pets might also act as a reservoir for the acquisition of such organisms in the community [18]. Misuse or overuse of antimicrobial drugs in Bosnia and Herzegovina, as well as the migratory flux of regional populations, could result in the emergence and selection of ESBL producers in the community [2,3].

In conclusion, this study showed that SHV-type and TEM-type ESBLs predominated among community isolates of *K. pneumoniae* and *Esch. coli*, respectively. This is in contrast to previous reports that CTX-M-type β-lactamases are predominant among *Esch. coli* community isolates [19]. Both TEM- and SHV-type β-lactamases are usually limited to nosocomial isolates of *Klebsiella* spp. [1,20]. This is the first report of the predominance of SHV-5 among community isolates. Since the isolates showed diverse pulsed-field gel electrophoresis patterns, it was concluded that clonal spread of bla_{TEM} and bla_{SHV} genes had not

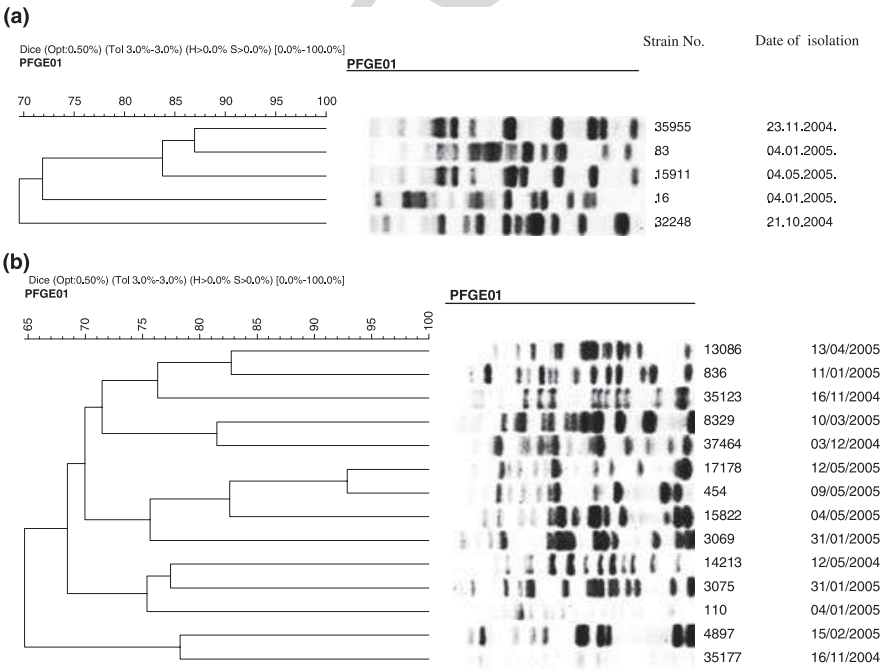

Fig. 1. Dendrograms obtained following pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested genomic DNA from 19 extended-spectrum β-lactamase-producing isolates of (a) *Escherichia coli* and (b) *Klebsiella pneumoniae*.

occurred, and that dissemination of the bla_{TEM} and bla_{SHV} genes was probably a result of genetic exchange of plasmids among different clones.

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