Emergence of Uropathogenic Extended-spectrum β Lactamases-producing *Escherichia coli* Strains in the Community

Tatjana Marijan[1], Jasmina Vraneš[1,2], Branka Bedenić[2,3], Ana Mlinarić-Džepina[1], Vanda Plečko[3] and Smilja Kalenić[2,3]

[1] Department of Microbiology, Zagreb Institute of Public Health, Zagreb, Croatia
[2] Department of Microbiology, Zagreb University Medical School, Zagreb, Croatia
[3] Department of Clinical and Molecular Microbiology, University Hospital Center Zagreb, Zagreb, Croatia

A B S T R A C T

The aim of this study was to determine the virulence characteristics and resistance pattern of the extended-spectrum β lactamases (ESBLs)-producing Escherichia coli strains isolated from urine of outpatients in the Zagreb region during a five-month period, and to compare them with the non ESBLs-producing E. coli strains isolated in the same period. Out of 2,451 E. coli strains isolated from urine of nonhospitalized patients with significant bacteriuria, a total of 39 ESBLs-producing strains (1.59%) were detected by a double-disk diffusion technique and by the broth-dilution minimal inhibitory concentration reduction method. The 45 non ESBLs-producing strains were randomly chosen, and phenotype of the two groups of strains was characterized and compared. Serogroup O4, hemolysin production, expression of P- and type 1 fimbriae as well as resistance to gentamicin and amikacin were significantly more prevalent characteristics among the ESBLs-producing strains than among non ESBLs-producing strains (p<0.01), while higher prevalence of trimethoprim-sulfamethoxazole resistance among ESBLs-producing strains was not statistically significant (p>0.05). Chromosomal DNA analysis by pulsed-field gel electrophoresis exhibited a great genomic similarity among ESBLs-producing strains and revealed that those highly virulent and resistant E. coli strains isolated from urine of outpatients in the Zagreb region had a clonal propagation.

Key words*: Urinary tract infections, Escherichia coli, virulence, extended-spectrum beta-lactamases, clonal dissemination*

Introduction

Recent data indicate that urinary tract infections caused by extended-spectrum β lactamases (ESBLs)-producing *Escherichia coli* may be an emerging problem in outpatient settings in various parts of the world. Possible community-acquisition of ESBLs-producing *E. coli* was first reported in 1998 from Ireland when a nalidixic acid resistant *E. coli* producing an ESBL was isolated from urine of an elderly patient who did not have a recent history of hospitalization[1]. Since than, ESBLs-producing *E. coli* have been increasingly recognized in the community[2–9].

An interesting issue concerning the epidemiology of ESBLs-producing organisms is the evolution, mainte-nance, and dissemination of resistance genes among bacterial populations in larger geographic regions[10]. Zagreb region has 1,200,000 inhabitants and besides Zagreb eight smaller towns are included in the region. Zagreb Institute of Public Health is the biggest regional institute of public health in Croatia, which collects samples for microbiological analysis from over 800 general practitioner offices. During a previous survey conducted over a five-month period in 2004 at the Zagreb Institute of Public Health, 39 of 2,451 consecutive, nonredundant *E. coli* strains deemed to cause urinary tract infections produced an ESBL. The aim of this study was to characterize those strains isolated from urine of nonhospitalized

patients, and to compare them with the characteristics of 45 randomly chosen non ESBLs-producing *E. coli* strains isolated from urine of nonhospitalized patients in the same period. The genetic relatedness of the ESBLs-producing isolates was analyzed by digesting genomic DNA with the restriction endonuclease *XbaI* and by performing pulse-field gel electrophoresis (PFGE).

Materials and Methods

Bacterial strains – origin and identification: During the five-month study period (January to May 2004) a total of 2,451 *E. coli* strains were isolated from urine of nonhospitalized patients with significant bacteriuria in the Department of Microbiology at Zagreb Institute of Public Health, Zagreb, Croatia. A total of 39 ESBLs-producing *E. coli* strains were detected and collected, as well as 45 randomly chosen non ESBLs strains. Only one isolate per patient was included. The *E. coli* isolates were identified by standard biochemical procedures and stored in deep-agar tubes at 4 °C (1.5% nutrient agar, Difco Lab., Detroit, MI, USA) and subcultured on tryptic soy agar (TSA, Difco Lab., Detroit, MI, USA) before use.

Antibiotic susceptibility testing: Antibiotic susceptibility testing was performed on Mueller-Hinton agar (Oxoid Ltd., Hampshire, UK) medium by a standard disk diffusion method with a panel of 17 antimicrobial drugs according to the recommendations of the National Committee for Clinical Laboratory Standards[11]. The tablet disks contained the following antimicrobial agents: amoxicillin, amoxicillin-clavulanic acid, piperacillin, piperacillin-tazobactam, cephalexin, cefuroxime, ceftazidime, ceftibuten, ceftriaxone, aztreonam, imipenem, gentamicin, netilmicin, amikacin, trimethoprim-sulfamethoxazole, ciprofloxacin, and nitrofurantoin. Strains with intermediate zones were considered resistant.

ESBL tests: The ESBL production was detected by double disk diffusion technique. In this test a plate was inoculated as for a standard disk diffusion test. Disk containing aztreonam and expanded-spectrum cephalosporins were than placed 30 mm from an amoxicillin-clavulanate disk prior to incubation. After overnight incubation at 37 °C the production of ESBLs was detected by the presence of characteristic distortions of the inhibition zones indicative of clavulanate potentiation of the activity of the test drug[12]. The broth-dilution minimal inhibitory concentration (MIC) reduction method was used as confirmatory test (>3-dilution reduction in the MIC of cefotaxime or ceftazidime in the presence of clavulanic acid in concentration of 2 mg/L)[13].

Serogroup determination: All *E. coli* isolates were serotyped using 17 different O-antisera (Institute of Immunology, Zagreb, Croatia), These O types (O1, O2, O4, O5, O6, O7, O8, O9, O11, O15, O17, O18, O20, O25, O50, O62 and O75) were selected because of their frequent occurrence as urinary pathogens. Serotyping was performed on glass slides and confirmed using a mechanized microtechnique[14].

Hemolytic activity: The production of α hemolysin was tested on human blood agar plates. The bacteria growing on TSA were stabbed with a sterile straight wire into 5% human blood agar. After 18 to 24 h of incubation at 37 °C, the clearing zone was observed.

Adhesins determination: The expression of adhesins was defined by hemagglutination and inhibition of hemagglutination in microtiter plates, as previously described[15,16]. Briefly, hemagglutination (HA) was performed using human erythrocytes and sheep, ox and guinea pig erythrocytes. Inhibition of HA was performed with P_1 antigen-containing pigeon egg white and with D-mannose (Sigma Chemical Co., St. Louis, USA), as previously described[15,17]. Isolates were considered to express P-fimbriae if HA was positive with human erythrocytes and inhibition of HA was positive with pigeon egg white, which was confirmed by agglutination of receptor-coated latex beads. The type 1 fimbriae were considered to be expressed if HA was positive with guinea pig erythrocytes. D-mannose always inhibited HA of guinea pig erythrocytes (mannose sensitive, MS HA), but it never inhibited HA of human, ox or sheep erythrocytes (mannose resistant, MR HA).Strains with MR HA ability and without detected P-specificity was considered to express X adhesin, and strains which agglutinated sheep and human blood group A erythrocytes, but did not agglutinate human blood group 0 erythrocytes, were considered to express 0NAP adhesin.

Sera and serum sensitivity assay: Blood was obtained by venipuncture from three healthy volunteers and was allowed to clot at room temperature for 30 min and overnight at 4 °C. After centrifugation at 1000 g for 15 min at 4 °C, serum was removed and pooled. A portion of the pooled serum was decomplemented by heating at 56°C for 30 min and used as test controls. Bacterial susceptibility to serum killing was measured by assessing regrowth after incubation in normal human serum according to Schiller and Hatch method[18]. Briefly, after adjustment of each bacterial suspension to a concentration equivalent to the concentration of 10^3 bacteria/mL, the mixture of 0.1 mL of bacterial suspension and 0.1 mL of pooled fresh human serum were incubated at 37 °C for two hours. Finally, the percent bacterial survival was determined by plating 0.1 mL samples onto Mueller-Hinton agar plates and the number of bacteria per milliliter was determined after incubation at 37 °C for 18–24 hours and compared to control which contained mixture of bacterial suspension and decomplemented serum. The bacterial strain was determined as serum sensitive (SS) if there were more than 90 percent killed bacteria regarded to serum action. Opposite, if there were less or equal to 90 percent killed bacteria, bacterial strain was determined as serum resistant (SR).

DNA macrorestriction and PFGE: Genomic DNA was prepared by a protocol devised from different methods published elsewhere[19,20]. Cleavage of the agarose-embedded DNA was achieved with 0.2 U/µL *XbaI* (Invitrogen) according to instructions of the manufacturer. PFGE was performed in the CHEF DRII System (Bio-Rad, Rich-

mond, CA, USA) under the following conditions: 0.5 x TBE, 1% agarose, 12 °C, 6V/cm. Run times and pulse times were 5–50 s for 22 h with linear ramping. The gels were stained with ethidium bromide (1 µg/mL) and photographed under UV light. The PFGE patterns were compared initially by visual comparison according to the guidelines of Tenover et al.[21]. Patterns were considered indistinguishable if every band was shared, closely re-

lated if they differed from one another by only three or fewer clearly visible bands, possibly related if they differed by 4–6, and different if they differed by seven or more bands. PFGE patterns were also analyzed with the GelCompar II computer software (Applied Maths, Sint-Martns-Latem, Belgium). Cluster analysis of the Dice similarity coefficients based on the unweighted pair group method using arithmetic averages was done to generate a

TABLE 1
CHARACTERISTICS OF 39 ESBLS-PRODUCING *E. COLI* STRAINS

Isolate No.	Age/ Gender[a]	Resistotype [b]	Serogroup[c]	Adhesins [d]	Hemolysin	SBA [e]
1	34/ f	NF	NT	P fimbriae	+	SS
2	65/ f	SXT	NT	HA=0	–	SS
3	76/ f	SXT, NF	NT	X+type 1 fimbriae	–	SS
4	30/ m	/	O4	P+type 1 fimbriae	+	SS
5	41/ f	/	O4	P+type 1 fimbriae	+	SS
6	2/ f	Gm, NET, AK	O6	type 1 fimbriae	+	SS
7	60/ f	/	O4	P+type 1 fimbriae	+	SS
8	15/ f	Gm, AK	O4	P+type 1 fimbriae	+	SS
9	50/ f	Gm, AK, SXT	O4	P+type 1 fimbriae	+	SR
10	1/ f	Gm, AK, SXT	O4	P+type 1 fimbriae	+	SS
11	28/ f	Gm, SXT	O4	P+type 1 fimbriae	+	SR
12	26/ f	Gm, NET, AK	O4	P+type 1 fimbriae	+	SS
13	78/ f	Gm, NET, AK, PTZ	O4	P+type 1 fimbriae	+	SS
14	1/ m	Gm, AK	O4	P+type 1 fimbriae	+	SS
15	79/ f	Gm, NET, AK	O4	P+type 1 fimbriae	+	SS
16	1/ f	Gm, AK	O4	P+type 1 fimbriae	+	SS
17	50/ f	Gm, AK	O4	P+type 1 fimbriae	+	SR
18	1/ f	Gm, AK	O4	P+type 1 fimbriae	+	SS
19	3/ m	Gm, AK	O4	P+type 1 fimbriae	+	SS
20	25/ f	Gm, NET, AK	O4	P+type 1 fimbriae	+	SS
21	74/ f	/	O4	P+type 1 fimbriae	+	SS
22	1/ m	Gm,AK	O4	P+type 1 fimbriae	+	SR
23	30/ f	Gm, AK	O4	P+type 1 fimbriae	+	SR
24	39/ f	Gm, AK	O4	P+type 1 fimbriae	+	SR
25	52/ f	Gm, SXT, NF, CIP	O6	HA=0	–	SR
26	39/ f	AMC, SXT, CIP	O1	HA=0	–	SS
27	58/ f	Gm, AK	O4	P+type 1 fimbriae	+	SS
28	36/ f	Gm, AK	O4	P+type 1 fimbriae	+	SR
29	29/ f	Gm, NET, AK	O4	P+type 1 fimbriae	+	SR
30	77/ f	Gm, NET, AK	O15	HA=0	–	SS
31	73/ f	Gm, NET, NF	O4	HA=0	–	SS
32	2/ f	Gm, NET, AK	O6	type 1 fimbriae	+	SS
33	52/ f	Gm, SXT, NF, CIP	O6	HA=0	–	SR
34	1/ m	Gm	NT	HA=0	–	SR
35	49/ m	Gm, AK	O4	P+type 1 fimbriae	–	SR
36	40/ f	Gm, NET, AK	O6	P+type 1 fimbriae	+	SS
37	70/ m	NF, CIP	O4	type 1 fimbriae	–	SS
38	67/ f	Gm, AK, SXT	NT	HA=0	–	SS
39	55/ m	Gm, AK	NT	P+type 1 fimbriae	+	SS

[a] m – masculine, f – feminine, [b] Gm – gentamicin, NET – netilmicin, AK – amikacin, AMC – amoxicillin-clavulanate, PTZ – piperacillin-tazobactam, SXT – trimethoprim-sulfamethoxazole, FM – nitrofurantoin, CIP – ciprofloxacin, [c] NT – nontypeable strain, [d] HA=0 – nonagglutinating strain, [e] SS – serum sensitive, SR – serum resistant

TABLE 2
CHARACTERISTICS OF 45 NON ESBLS-PRODUCING *E. COLI* STRAINS

Isolate No.	Age/ Gender[a]	Resistotype [b]	Serogroup [c]	Adhesins [d]	Hemolysin	SBA [e]
1	52/ f	AMX, CN	O75	X+type 1 fimbriae	+	SR
2	52/ f	/	NT	HA=0	–	SS
3	27/ f	/	O18	X adhesin	+	SR
4	72/ f	AMX, SXT	O17	X adhesin	–	SS
5	54/ m	AMX	O2	P fimbriae	+	SS
6	61/ f	/	O1	0NAP adhesin	–	SR
7	55/ f	/	O1	X adhesin	–	SS
8	77/ f	AMX, NF	NT	HA=0	–	SS
9	73/ f	/	O15	X adhesin	–	SS
10	73/ f	AMX	O9	X adhesin	–	SS
11	89/ f	AMX, SXT	NT	P fimbriae	–	SS
12	71/ f	/	O2	P fimbriae	+	SS
13	7 / m	AMX	O6	HA=0	+	SS
14	65/ f	AMX, SXT	NT	type 1 fimbriae	–	SS
15	56/ f	/	O2	P+type 1 fimbriae	+	SR
16	93/ f	/	O2	HA=0	–	SS
17	34/ f	CN	O6	HA=0	+	SR
18	61/ f	CN	O25	HA=0	–	SR
19	26/ f	AMX	O18	P+type 1 fimbriae	+	SR
20	61/ f	SXT	O2	P+type 1 fimbriae	+	SR
21	81/ f	AMX	O18	P fimbriae	+	SR
22	81/ f	AMX, SXT	NT	P fimbriae	–	SS
23	21/ f	/	O6	X adhesin	+	SR
24	32/ f	/	O2	P+type 1 fimbriae	+	SR
25	42/ m	/	O6	HA=0	+	SR
26	16/ f	/	O75	X+type 1 fimbriae	+	SR
27	56/ f	/	O18	HA=0	–	SR
28	74/ m	/	O2	HA=0	–	SS
29	33/ f	CIP	O17	HA=0	–	SS
30	80/ f	/	O2	HA=0	–	SR
31	71/ f	/	NT	P fimbriae	–	SS
32	? / f	/	NT	type 1 fimbriae	+	SR
33	34/ f	/	NT	P+type 1 fimbriae	–	SR
34	37/ f	SXT	O25	X+type 1 fimbriae	+	SR
35	32/ f	/	NT	HA=0	–	SS
36	67/ f	/	O7	X adhesin	–	SR
37	53/ f	CN	O75	X+type 1 fimbriae	+	SR
38	78/ m	/	NT	X adhesin	–	SR
39	54/ f	AMX	NT	type 1 fimbriae	–	SR
40	81/ f	/	NT	HA=0	–	SS
41	68/ f	/	NT	HA=0	–	SS
42	80/ f	/	NT	P fimbriae	+	SS
43	77/ m	AMX	NT	HA=0	–	SR
44	75/ f	/	O18	type 1 fimbriae	–	SR
45	78/ f	AMX, CN, SXT	NT	type 1 fimbriae	–	SR

[a] m – masculine, f – feminine, [b] AMX – amoxicillin, CN – cephalexin, SXT – trimethoprim-sulfamethoxazole, NF – nitrofurantoin, CIP – ciprofloxacin, [c] NT – nontypeable strain, [d] HA=0 – nonagglutinating strain, 0NAP – 0 negative A positive, [e] SS – serum sensitive, SR – serum resistant

dendrogram describing the relationship among *E. coli* pulsotypes. Isolates were considered to be identical if they showed 100% similarity and were considered clo-

nally related if they showed greater than 80% similarity (comparable to the three or fewer fragment difference already noted).

Statistical methods: Proportions were compared by the χ^2-test and by Fisher's exact test when the number in any of the 2x2 table was ≤5. A p-value <0.01 was considered statistically significant.

Results

The characteristics of 39 community-acquired ESBLs-producing *E. coli* strains isolated in the 5-month study period are presented in Table 1. Among 39 ESBLs-producing strains tested, co-resistance to various antimicrobial agents was observed in 35 strains, such as resistance to gentamicin, netilmicin, amikacin, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, nitrofurantoin, and ciprofloxacin. The co-resistance to gentamicin was the most frequently observed, followed by resistance to amikacin, netilmicin and trimethoprim-sulfamethoxazole. The most frequently detected serogroup was O4, detected in 26 out of 39 investigated strains. High virulence capacity of the strains based on adhesins expression and hemolytic activity was observed, and 28 strains produced α hemolysin, 27 strains expressed P-fimbriae, while 30 strains expressed type 1 fimbriae. In only eight strains adhesins expression was not detected. Most strains (27) were sensitive to bactericidal activity of normal human serum, and were isolated from urine of female patients. The 8 male and 31 female patients ranged in age from 1 to 79 years (median age 39 years).

The characteristics of 45 randomly chosen non ESBLs-producing *E. coli* strains isolated from urine of nonhospitalized patients in the same period are presented in Table 2. Most strains were sensitive to all antibiotics tested, while 14 strains were resistant to amoxicillin and seven to trimethoprim-sulfamethoxazole. There was no significantly higher frequency among serogroups distribution, and 16 strains were not typeable with the diagnostic sera used. Type 1 fimbriae were expressed in 14 strains, P fimbriae in 12 strains, and in15 strains adhesins were not detected. Hemolysin production was observed in 18 strains, and 25 strains were resistant to serum bactericidal activity. The strains were isolated from urine of six male and 39 female patients ranged in age from 7–93 years (median age 58 years).

Molecular characterization of 39 ESBLs-producing strains performed by PFGE revealed genetic relatedness between 25 strains (>80% similarity). After the extraction of genomic DNA and digestion with *XbaI* restriction enzyme, the PFGE fingerprints exposed the existence of two clusters (Figure 1), which were closely related, resulting in one to three band differences (the Dice similarity coefficient, Dsc. ≥83.49%). The first cluster was composed of 11 strains (≥85.48% of similarity) and the second cluster was composed of 14 strains (≥87.99% of similarity). The rest of 14 ESBLs-producing strains tested were not clonally related (Dsc. <80%, Figure 1).

Serogroup O4, hemolysin production, expression of P- and type 1 fimbriae as well as resistance to gentamicin and amikacin were significantly more prevalent characteristics among the ESBLs-producing strains than among
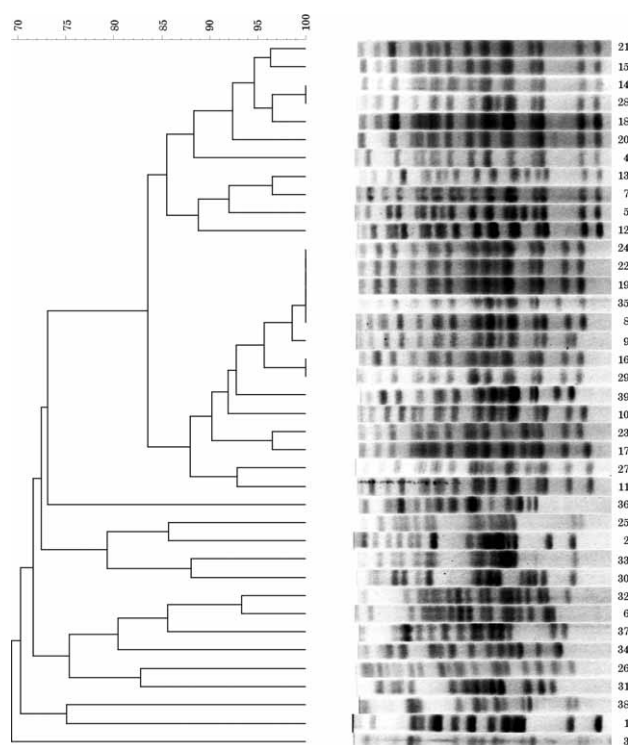

Fig. 1. Dendrogram and PFGE fingerprints of 39 ESBL-producing E.coli isolates after digestion with XbaI restriction enzyme. The Dice similarity coefficient (Dsc.) for strains 21, 15, 14, 28, 18, 20, 4, 13, 7, 5, 12, 24, 22, 19, 35, 8, 9, 16, 29, 39, 10, 23, 17, 27 and 11≥83.49%. Other strains are not clonally related (Dsc.<80%).

non ESBLs-producing strains (p<0.01), while higher prevalence of trimethoprim-sulfamethoxazole resistance among ESBLs-producing strains was not statistically significant (p>0.05, Table 3). The difference in serum sensitivity between the two groups of strains was not statistically significant as well (p>0.01).

Discussion

The present study demonstrated clonal dissemination of highly virulent ESBLs-producing *E. coli* strains isolated from urine of nonhospitalized patients in a large, well defined region in Croatia. Preliminary results of molecular characterization have shown production of SHV class of ESBL, and further analysis follows (data not presented).

Although urinary tract infection is not usually thought of as a disease associated with community-wide outbreaks, certain multidrug-resistant, uropathogenic lineages of *E. coli* have exhibited epidemic behavior[22]. *E. coli* O15:K52:H1 caused an outbreak of community-acquired cystitis, pyelonephritis, and septicemia in south London, England in 1986 to 1987, when strains of this serotype expressed P fimbriae, produced aerobactin, and displayed an unusual multiple antimicrobial resistance phenotype[22]. The subsequent recognition of O15:K52:H1

as the second most common serotype among *E. coli* bacteriemia isolates at a Copenhagen hospital (originated from urinary tract), together with the observation that Copenhagen isolates exhibited the same virulence factors as the south London outbreak strains, provided further evidence of the pathogenic potential of *E. coli* O15:K52:H1 and suggested that this serotype might constitute a widespread virulent clone[23]. This was confirmed by the findings from Spain, which indicated that *E. coli* O15:K52:H1 constitutes a broadly distributed and clinically significant uropathogenic clone with fluid antimicrobial resistance capabilities, and is an endemic cause of urinary tract infection in Barcelona[24]. In 2001 Manges and coworkers reported that a single clonal group accounted for nearly half of community-acquired urinary tract infections in women that were caused by *E. coli* strains with resistance to trimethoprim-sulfamethoxazole in three geographically diverse communities in the United States of America[25]. Subsequently, those strains were signated as a clonal group A (CGA) and it was found that they exhibit a robust virulence profile suggesting enhanced extraintestinal virulence[26,27]. Johnson and coworkers concluded that this combination of resistance and virulence may account for CGA's recent emergence as a broadly disseminated epidemic clone[26]. In the present study, the observed high virulence of clonally related, multiresistant *E. coli* strains isolated from urine of non-hospitalized patients in the Zagreb region causes concern and requires additional surveillance of the clone spread in the community.

A number of risk factors have been identified as linked with the acquisition of community-acquired infections involving ESBLs-positive isolates. Mostly, a previous hospitalization or antibiotic therapy within the previous three months, old age (>60 years), male gender, confinement to bed with debilitation and urinary catheterization were detected as risk factors[28,29]. In the present study, females had significantly higher rates of acquisition of ESBLs-producing *E. coli* strains and median age of patients was only 39 years. Moreover, patients were not institutionalized and lived in different parts of the region. Limitations of the study include the paucity of clinical and epidemiological data, therefore no conclusions about risk factors, spread or origin of the strains can not be made. Further studies are warranted to investigate whether community-onset urinary tract infections caused by ESBLs-producing, highly virulent *E. coli* strains are related to a point source, or transmission within the community. Recent intriguing hypothesis suggests possibility that multidrug-resistant, uropathogenic *E. coli* strains may be spread by contaminated food products[30].

Conclusion

PFGE highlighted that highly virulent uropathogenic ESBLs-producing *E. coli* strains isolated from outpatients in Zagreb region had a clonal propagation. Further molecular characterization of those strains is needed, as well as the additional epidemiological studies of the strains origin and spread in the community.

Acknowledgements

REFERENCES

1. CORMICAN M, MORRIS D, CORRBET-FEENEY G, FLYNN J, Diagn Microbiol Infect Dis, 32 (1998) 317. — 2. COLODNER R, KENESS Y, CHAZAN B, RAZ R, Int J Antimicrob Agents 18 (2001) 189. — 3. BOU G, CARTELLE M, TOMAS M, CANLE D, MOLINA F, MOURE R, EIROS JM, GUERRERO A, J Clin Microbiol, 40 (2002) 4030. — 4. ARPIN C, DUBOIS V, COULANGE L, ANDRE C, FISCHER I, NOURY P, GROBOST F, BROCHET J-P, JULLIN J, DUTILH B, LARRIBET G, LAGRANGE I, QUENTIN I, Antimicrob Agents Chemother, 47 (2003) 3506. — 5. LESCURE FX, EVEILLARD M, DOUADI Y, EB F, J Hosp Infect, 49 (2001) 149. — 6. RODRIGUEZ-BANO J, NAVARRO MD, ROMERO L, MARTINEZ-MARTINEZ L, MUNIAIN M, PEREA EJ, PEREZ-CANO R, PASCUAL A, J Clin Microbiol, 42 (2004) 1089. — 7. MUNDAY CJ, WHITEHEAD GM, TODD NJ, CAMPBELL M, HAWKEY PM, J Antimicrob Chemother, 54 (2004) 735. — 8. WOODFORD N, WARD ME, KAUFMANN ME, TURTON J, FAGAN EJ, JAMES D, JOHNSON AP, PIKE R, WARNER M, CHEASTY T, PEARSON A, HARRY S, LEACH JB, LOUGHREY A, LOWERS JA, WARREN JA, LIVERMORE DM, J Antimicrob Chemother, 54 (2004) 735. — 9. PITOUT JDD, NORDMANN P, LAUPLAND KB, POIREL L, J Antimicrob Chemother, 56 (2005) 52. — 10. LIVERMORE DM, Clin Infect Dis, 36 (2003) S11. — 11. NCCLS, Performance standards for antimicrobial susceptibility testing: approved standard (NCCLS, Wayne, 1997–2002). — 12. JARLIER V, NICOLAS M-H, FURNIER G, PHILIPPON A, Rev Infect Dis, 10 (1988) 867. — 13. NCCLS, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard (NCCLS, Wayne, 1997–2002). — 14. ORSKOV F, ORSKOV I, Methods Microbiol, 14 (1984) 43. — 15. VRANES J, APMIS, 105 (1997) 831. — 16. VRANES J, KRUZIC V, STERK-KUZMANOVIC N, SCHOENWALD S, Infection, 31 (2003) 216. — 17. JOHNSON JR, SWANSON JL, NEILL MA, Infect Immun, 60 (1992) 578. — 18. SCHILLER NL, HATCH RA, Diagn Microbiol Infect Dis, 1 (1983) 145. — 19. MATUSHEK MG, BONTEN MJM, HAYDEN MK, J Clin Microbilol, 34 (1996) 2598. — 20. STRUELENS MJ, ROST F, DEPLANO A, MAAS A, SCHWAM V, SERRUYS E, CREMER M, Am J Med, 95 (1993) 489. — 21. TENOVER FC, ARBEIT RD, GOERING RV, MICKELSEN PA, MURRAY BE, PERSING DH, SWAMINATHAN B, J Clin Microbiol, 33 (1995) 2233. — 22. PHILLIPS I, EYKYN S, KING A, GRANDSEN WR, ROWE B, FROST JA, GROSS RJ, Lancet, 1 (1988) 1038. — 23. OLSEN B, KOLMOS HJ, ORSKOV F, ORSKOV I, J Hosp Infect, 31 (1995) 295. — 24. PRATS G, NAVARRO F, MIRELIS B, DALMAU D, MARGALL N, COLL P, STELL A, JOHNSON JR, J Clin Microbiol, 38 (2000) 201. — 25. MANGES AR, JOHNSON JR, FOXMAN B, BRYAN TT, FULLERTON KE, RILEY LW, N Engl J Med, 345 (2001) 1007. — 26. JOHNSON JR, MURRAY AC, KUSKOWSKI MA, SCHUBERT S, PRERE M-F, PICARD B, COLODNER R, RAZ R, Emerg Infect Dis, 11 (2005) 141. — 27. MANGES AR, DIETRICH PS, RILEY LW, Clin Infect Dis, 38 (2004) 329. — 28. DAZA R, GUTIERREZ J, PIEDROLA G, Int J Antimicrob Agents, 18 (2001) 211. — 29. COLODNER R, ROCK W, CHAZAN B, KELLER N, GUY N, SAKRAN W, RAZ R, Eur J Clin Microbiol Infect Dis, 23 (2004) 163. — 30. RAMCHANDANI M, MANGES AR, DEBROY CR, SMITH SP, JOHNSON JR, RILEY LW, Clin Infect Dis, 40 (2005) 251.

J. Vraneš

Zagreb Institute of Public Health, Mirogojska Road 16, 10000 Zagreb, Croatia
e-mail: jasmina.vranes@publichealth-zagreb.hr

POJAVA UROPATOGENIH SOJEVA BAKTERIJE *ESCHERICHIA COLI* KOJI PRODUCIRAJU β LAKTAMAZE PROŠIRENOG SPEKTRA U ZAJEDNICI

S A Ž E T A K

   Cilj ovog istraživanja bio je utvrditi virulentne značajke i rezistotip sojeva bakterije *Escherichia coli* koji produciraju β laktamaze proširenog spektra, izoliranih iz mokraće ambulantnih pacijenata zagrebačke regije u razdoblju od pet mjeseci, i usporediti ih sa sojevima *E. coli* koji ne stvaraju β laktamaze proširenog spektra, a izolirani su u istom razdoblju. Od 2451 *E. coli* sojeva izoliranih iz mokraće izvanbolničkih pacijenata sa signifikantnom bakteriurijom, ukupno 39 sojeva produciralo je β laktamaze proširenog spektra (1,59%), što je detektirano metodom dvostruke disk-difuzije i metodom redukcije minimalne inhibicijske koncentracije u bujonu. Slučajnim odabirom prikupljeno je 45 sojeva koji ne produciraju β laktamaze proširenog spektra, te je određen i uspoređen fenotip ove dvije grupe sojeva. Najzastupljenije značajke sojeva koji su producirali β laktamaze proširenog spektra bile su serogrupa O4, produkcija hemolizina, ispoljavanje P i tip 1 fimbrija, te rezistencija na gentamicin i amikacin (p<0,01), dok veća zastupljenost rezistencije na trimetoprim-sulfametoksazol nije bila statistički značajna (p>0,05). Kromosomska DNA analiza pomoću gel-elektroforeze u izmjeničnom polju pokazala je veliku genetsku sličnost među sojevima koji produciraju β laktamaze proširenog spektra i otkrila klonalno širenje ovih visoko virulentnih i rezistentnih uropatogenih sojeva *E. coli* među izvanbolničkim pacijentima zagrebačke regije.