ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2011 Vol. 54, Št. 2: 5–13

Genetic background of uropathogenic *Escherichia coli* isolates from Slovenia in relation to fluoroquinolone and sulfamethoxazole/trimethoprim resistance

Genetsko ozadje uropatogenih sevov bakterije *Escherichia coli* iz Slovenije v povezavi z odpornostjo proti fluorokinolonom in sulfametoksazol/trimetoprimu

[1]Marjanca Starčič Erjavec, [2]Anja Palandačić, [1]Darja Žgur-Bertok, [1]Jerneja Ambrožič Avguštin*

[1]Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia
[2]Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Groblje 3, 1230 Domžale, Slovenia
* correspondence: jerneja.ambrozic@bf.uni-lj.si

Abstract: A total of 99 *E. coli* urinary tract isolates were investigated for phylogenetic groups and 21 virulence related genes in relation to fluoroquinolone and sulfamethoxazole/trimethoprim resistance. We found that the B2 group was by far the most prevalent among susceptible isolates, while resistant isolates were more evenly distributed among groups A, B2 and D. Isolates from the B2 group exhibited the highest prevalence of virulence factors. Virulence genes *hlyA*, *iroN* and *kpsMTII* were statistically associated with fluoroquinolone susceptible isolates and *picU* with sulfamethoxazole/trimethoprim susceptible isolates. Fluoroquinolone susceptible isolates of the phylogenetic group A were significantly associated with genes *papGII*, *kpsMTII* and *iss* and the susceptible group B_2 isolates with genes *hra* in *iroN*. Among isolates susceptible to sulfamethoxazole/trimethoprim the presence of the *hra* gene was statistically significantly associated with phylogenetic group B2, while among resistant isolates, *papGII* was associated with phylogenetic group D.

Keywords: *Escherichia coli*, urinary tract, phylogenetic groups, virulence trait, fluoroquinolone resistance, sulfamethoxazole/trimethoprim resistance

Izvleček: V naši raziskavi smo 99 uropatogenih izolatov *E. coli* uvrstili v filogenetske skupine in pri vsakem preverili prisotnost 21-ih genov povezanih z virulenco ter podatke analizirali v povezavi z odpornostjo izolata za fluorokinolone in sulfametoksazol/trimetoprim. Ugotovili smo, da se izolati, ki so občutljivi za fluorokinolone in /ali sulfametoksazol/trimetoprim uvrščajo predvsem v filogenetsko skupino B2, odporni izolati pa v približno enakih deležih v skupine A, B2 in D. Izolati v filogenetski skupini B2 so imeli največ genskih zapisov za virulentne dejavnike. Izolati občutljivi za fluorokinolone so imeli statistično značilno pogosteje preučevane genske zapise *hlyA*, *iroN* in *kpsMTII* v primerjavi z odpornimi izolati, medtem ko so imeli izolati občutljivi za sulfametoksazol/trimetoprim v primerjavi z odpornimi izolati statistično značilno pogosteje genski zapis za *picU*. Pri izolatih, ki so bili občutljivi za fluorokinolone, smo ugotovili statistično značilne povezave med prisotnostjo genov *papGII*, *kpsMTII* ter *iss* in uvrstitvijo izolata v filogenetsko skupino A ter genov *hra* in *iroN* ter uvrstitvijo

izolata v filogenetsko skupino B2. Pri izolatih, ki so bili občutljivi za sulfametoksazol/trimetoprim, je bila statistično značilna povezava med prisotnostjo gena *hra* in uvrstitvijo izolata v filogenetsko skupino B2, pri odpornih izolatih pa je bil gen *papGII* statistično značilno povezan z uvrstitvijo izolata v filogenetsko skupino D.

Ključne besede: *Escherichia coli*, sečila, filogenetske skupine, virulentni dejavniki, odpornost proti fluorokinolonom, odpornost proti trimetoprimu in sulfametoksazolu

Introduction

Urinary tract infections (UTIs) are one of the most frequent infectious diseases encountered in the developed world. Uropathogenic *Escherichia coli* (*E. coli*) strains (UPEC) are the major cause of uncomplicated UTI worldwide. In Slovenia *E. coli* causes approximately 80% of all UTIs (Lindič 2005). In comparison to commensal *E. coli* strains, UPEC possess an array of virulence factors namely, adhesins, toxins, polysaccharide coatings, invasins, iron uptake systems and systems to evade the host immune response (Oelschlaeger et al. 2002). UPEC mainly belong to the B2 phylogenetic group and to a lesser extent to the D group, while commensal strains belong to groups A and B1 (Picard et al. 1999). The most frequently prescribed drugs for the treatment of UTIs in general practices in Slovenia are trimethoprim/sulfamethoxazole (57% of prescribed antibiotics) and the fluoroquinolones norfloxacin and ciprofloxacin (38% of prescribed antibiotics) (Car et al. 2003). However, a major problem in treatment of UTIs is the emergence of *E. coli* strains resistant to these first-line antimicrobials. To unravel the relationship between resistance and virulence, several studies have dealt with the characteristics of fluoroquinolone and/or trimethoprim/sulfamethoxazole resistant strains, including phylogenetic background (A, B1, B2 and D group) and virulence factors (Drews et al. 2005, Horcajada et al. 2005, Johnson et al. 2003, Johnson et al. 2005, Johnson et al. 2009, Moreno et al. 2006, Piatti et al. 2008, Takahashi et al. 2009, Vila et al. 2002). Since no comparable data are available for UPEC isolates from Slovenia, we investigated the distribution of virulence genes among phylogenetic groups in relation to fluoroquinolone and sulfamethoxazole/trimethoprim resistance.

Material and methods

Bacterial strains

The UPEC isolates investigated in this study were collected and identified at the Institute of Public Health of the Republic of Slovenia (IVZ) between the years 2004–2007. The strains were isolated from urine of outpatients with cystitis, who sought help at general practises in Slovenian Community Health Centres. Only one isolate per patient was included in our study. A random sample of 45 ciprofloxacin resistant and 54 ciprofloxacin susceptible isolates, as determined by the disk diffusion method and interpreted according to the CLSI standards (Clinical and Laboratory Standards Institute 2007), were included in the study. Additionally all isolates were also tested for sulfamethoxazole/trimethoprim resistance.

Detection of phylogenetic groups and virulence factors

DNA to be PCR amplified for detection of phylogenetic groups and virulence factors was released from whole bacterial cells by boiling according to Le Bouguenec et al. (1992). For all isolates the phylogenetic groups (A, B1, B2 and D) were determined using the triplex PCR described by Clermont et al. (2000). Further, all isolates were screened for the presence of 21 urovirulence genes, including fimbriae/adhesins (*fimH* – type 1-fimbrial adhesin, *papGII* – P-fimbrial adhesin II, *sfaDE* – S-fimbriae, *bmaE* – M-fimbrial adhesin, *gafD* – G-fimbrial adhesin, *iha* – non fimbrial adhesin Iha and *hra* – non fimbrial adhesin Hra), toxins/autotransporters (*hlyA* – hemolysin A, *hbp* – haemoglobin protease, *sat* – secreted autotransporter toxin, *vat* – vacuolating autotransporter toxin and *picU* – autotransporter involved in intestinal colonization PicU), invasins (*ompA* – outer membrane

protein A, *ibeA* – invasion of brain endothelium and *aslA* – arylsulphatase-like protein), genes involved in iron acquisition (*iucD* – aerobactin synthesis, *iroN* – catecholate siderophore receptor and *irp2* – yersiniabactin biosynthesis), capsule synthesis (*kpsMTII*), increased serum survival (*iss*) and uropathogenic specific protein (*usp*). The employed primers are available at http://www.bf.uni-lj.si/fileadmin/users/1/biologija/genetika/Table-PCR-primers.pdf.

Statistical analysis

The significance of the results was established using the Fisher's exact test (2-tailed) available on-line on the web site http://www.langsrud.com/fisher.htm. The threshold for statistical significance was set at a P value < 0.05.

Results

Prevalence of phylogenetic groups and virulence factors in relation to resistance phenotypes

As seen from Table 1, the majority (30 out of 54, 56%) of the fluoroquinolone-susceptible strains were assigned to the phylogenetic group B2, followed by the D group with 17 strains (31%). The fluoroquinolone-resistant strains were evenly distributed among the phylogenetic groups A, B2 and D; 13 strains (29%) belonged to the A group, 14 strains (31%) to the B2 group and 16 strains (35%) to the D group. The differences between the prevalence of fluoroquinolone-susceptible and resistant strains in the A and B2 groups were statistically significant. Of the examined virulence genes 13 occurred with a higher prevalence among fluoroquinolone susceptible isolates while the associations of *hlyA*, *iroN* and *kpsMTII* with susceptibility were statistically significant. In accordance with the higher prevalence of the majority of virulence genes, the average virulence score among susceptible strains was higher compared to resistant strains (7.76 versus 6.13). The majority (26 out of 48, 54%) of the sulfamethoxazole/trimethoprim-susceptible strains were assigned to the phylogenetic group B2, followed by the D group with 15 strains (31%). The sulfamethoxazole/trimethoprim-resistant strains were distributed more evenly among the phylogenetic groups A (25%), B2 (35%) and D (35%). However, the differences between the number of sulfamethoxazole/trimethoprim-susceptible and resistant strains with regard to phylogenetic group was statistically not significant. Twelve of the examined virulence genes occurred with a higher prevalence among sulfamethoxazole/trimethoprim-susceptible isolates (Table 1), but only the occurrence of *picU* was statistically significant. The average virulence score among sulfamethoxazole/trimethoprim susceptible and resistant isolates was 7.31 versus 6.75.

Significant associations of virulence genes and phylogenetic groups in relation to resistance phenotypes

Only virulence genes with a prevalence of $\geq 10\%$ were selected for the analysis of associations of virulence genes and phylogenetic groups in relation to resistance phenotypes (Table 2).

While among fluoroquinolone and sulfamethoxazole/trimethoprim-susceptible strains the majority of the virulence genes were detected in isolates of the B2 group, only the occurrence of *hra* and *iroN* was statistically significant, the first among fluoroquinolone and sulfamethoxazole/trimethoprim-susceptible isolates and the second only among sulfamethoxazole/trimethoprim-susceptible isolates (Table 2). Among the latter isolates *papGII, kpsMTII* and *iss* were significantly associated with phylogenetic group A. Among sulfamethoxazole/trimethoprim-resistant isolates only *papGII* was significantly associated with the D group isolates (Table 2).

Discussion

This study showed that 56% of fluoroquinolone-susceptible and 54% of sulfamethoxazole/trimethoprim-susceptible isolates belonged to phylogenetic group B2, while fluoroquinolone- and sulfamethoxazole/trimethoprim-resistant isolates were evenly distributed among groups A, B2, and D. However, the percentage of fluoroquinolone-resistant isolates belonging to group B2 has been increasing in the last decade. Studies on *E. coli* isolated from 1998 to 2003 reported 12% (Johnson

Table 1: Prevalence of phylogenetic groups and virulence genes in relation to resistance phenotypes among the studied *E. coli* isolates

Tabela 1: Prevalence filogenetskih skupin in genov za dejavnike virulence pri odpornih in občutljivih izolatih *E.coli*

	Prevalence [N (%)]					
	FQ			SXT		
	S	R	P	S	R	P
	(N = 54)	(N = 45)		(N = 48)	(N = 51)	
Phylogenetic group						
A	4 (7)	13 (29)	0.007	4 (8)	13 (25)	ns
B1	3 (6)	2 (4)	ns	3 (6)	2 (4)	ns
B2	30 (56)	14 (31)	0.016	26 (54)	18 (35)	ns
D	17 (31)	16 (35)	ns	15 (31)	18 (35)	ns
Virulence gene						
fimH	52 (96)	45 (100)	ns	47 (98)	50 (98)	ns
papGII	25 (46)	17 (38)	ns	19 (40)	23 (45)	ns
sfaDE	20 (37)	10 (22)	ns	19 (40)	11 (22)	ns
bmaE	2 (4)	0 (0)	ns	0 (0)	2 (4)	ns
gafD	0 (0)	0 (0)	ns	0 (0)	0 (0)	ns
iha	21 (39)	18 (40)	ns	14 (29)	25 (49)	ns
hra	16 (30)	7 (16)	ns	14 (29)	9 (18)	ns
hlyA	10 (19)	0 (0)	0.002	6 (13)	4 (8)	ns
hbp	2 (4)	3 (7)	ns	3 (6)	2 (4)	ns
sat	14 (26)	11 (24)	ns	11 (23)	14 (27)	ns
vat	6 (11)	2 (4)	ns	6 (13)	2 (4)	ns
picU	14 (26)	5 (11)	ns	15 (31)	4 (8)	0.005
ompA	49 (91)	41 (91)	ns	43 (90)	47 (92)	ns
ibeA	8 (15)	8 (18)	ns	8 (17)	8 (16)	ns
aslA	0 (0)	0 (0)	ns	0 (0)	0 (0)	ns
iucD	32 (59)	29 (64)	ns	25 (52)	36 (71)	ns
iroN	37 (69)	17 (38)	0.003	29 (60)	25 (49)	ns
irp2	44 (81)	30 (67)	ns	40 (83)	34 (67)	ns
kpsMTII	39 (72)	19 (42)	0.004	32 (67)	26 (51)	ns
iss	12 (22)	8 (18)	ns	7 (15)	13 (25)	ns
usp	16 (30)	6 (13)	ns	13 (27)	9 (18)	ns
AVS	7.76	6.13		7.31	6.75	

FQ: Fluoroquinolone; Sxt: Sulfamethoxazole/Trimethoprim; S: Susceptible; R: Resistant; AVS: average virulence score (the average virulence score was calculated as the sum of all detected virulence associated genes divided with the number of isolates per group)/; ns – not statistically significant

Table 2: Associations of virulence genes with phylogenetic groups in relation to resistance phenotypes among the studied *E. coli* isolates.
Tabela 2: Povezava genov z zapisom za dejavnike virulence z uvrstitvijo v filogenetsko skupino in odpornostjo.

		Prevalence [no. (%)]									
		FQ				SXT					
		S		R		**P**	S		R		**P**
Virulence gene	Phylogenetic group										
fimH	A	4	(100)	13	(100)	ns	4	(100)	13	(100)	ns
	B1	3	(100)	2	(100)	ns	3	(100)	2	(100)	ns
	$B2_3$	29	(97)	14	(100)	ns	26	(100)	17	(94)	ns
	D	16	(94)	16	(100)	ns	14	(93)	18	(100)	ns
papGII	A	3	(75)	0	(0)	0,006	2	(50)	1	(8)	ns
	B1	1	(33)	0	(0)	ns	1	(33)	0	(0)	ns
	$B2_3$	13	(43)	5	(36)	ns	11	(42)	7	(39)	ns
	D	8	(47)	12	(75)	ns	5	(33)	15	(83)	0,005
sfaDE	A	1	(25)	1	(100)	ns	0	(0)	2	(15)	ns
	B1	0	(0)	0	(0)	ns	0	(0)	0	(0)	ns
	$B2_3$	15	(50)	6	(43)	ns	15	(58)	6	(33)	ns
	D	4	(24)	3	(19)	ns	4	(27)	3	(21)	ns
bmaE	A	0	(0)	0	(0)	ns	0	(0)	0	(0)	ns
	B1	1	(33)	0	(0)	ns	0	(0)	1	(50)	ns
	$B2_3$	0	(0)	0	(0)	ns	0	(0)	0	(0)	ns
	D	1	(11)	0	(0)	ns	0	(0)	1	(7)	ns
iha	A	1	(25)	4	(33)	ns	0	(0)	5	(42)	ns
	B1	0	(0)	0	(0)	ns	0	(0)	0	(0)	ns
	$B2_3$	12	(40)	8	(57)	ns	9	(35)	11	(61)	ns
	D	8	(47)	6	(38)	ns	5	(33)	9	(50)	ns
hra	A	0	(0)	4	(33)	ns	0	(0)	4	(33)	ns
	B1	0	(0)	0	(0)	ns	0	(0)	0	(0)	ns
	$B2_3$	13	(43)	1	(7)	0,019	12	(46)	2	(11)	0,021
	D	3	(18)	2	(14)	ns	2	(33)	3	(21)	ns
hlyA	A	0	(0)	0	(0)	ns	0	(0)	0	(0)	ns
	B1	0	(0)	0	(0)	ns	0	(0)	0	(0)	ns
	$B2_3$	7	(23)	0	(0)	ns	4	(15)	3	(17)	ns
	D	3	(38)	0	(0)	ns	2	(33)	1	(25)	ns
hbp	A	0	(0)	1	(8)	ns	1	(25)	0	(0)	ns
	B1	0	(0)	1	(50)	ns	0	(0)	1	(50)	ns
	$B2_3$	2	(7)	0	(0)	ns	1	(4)	1	(6)	ns
	D	0	(0)	1	(7)	ns	1	(11)	0	(0)	ns
sat	A	1	(25)	0	(0)	ns	0	(0)	1	(8)	ns
	B1	0	(0)	0	(0)	ns	0	(0)	0	(0)	ns
	$B2_3$	8	(27)	7	(50)	ns	7	(27)	8	(44)	ns
	D	5	(29)	4	(29)	ns	4	(44)	5	(28)	ns

		Prevalence [no. (%)]								
		FQ				SXT				
		S		R		**P**	S		R		P
Virulence gene	Phylogenetic group									
vat	A	0	(0)	1	(8)	ns	0	(0)	1	(8)	ns
	B1	0	(0)	0	(0)	ns	0	(0)	0	(0)	ns
	$B2_3$	6	(20)	1	(7)	ns	6	(23)	1	(6)	ns
	D	0	(0)	0	(0)	ns	0	(0)	0	(0)	ns
picU	A	2	(50)	1	(8)	ns	2	(50)	1	(8)	ns
	B1	0	(0)	0	(0)	ns	0	(0)	0	(0)	ns
	$B2_3$	9	(30)	4	(29)	ns	10	(38)	3	(17)	ns
	D	3	(18)	0	(0)	ns	3	(20)	0	(0)	ns
ompA	A	3	(75)	10	(77)	ns	2	(50)	11	(85)	ns
	B1	1	(33)	1	(50)	ns	1	(33)	1	(50)	ns
	$B2_3$	28	(93)	14	(100)	ns	25	(96)	17	(96)	ns
	D	17	(100)	16	(100)	ns	15	(100)	18	(100)	ns
ibeA	A	0	(0)	1	(8)	ns	0	(0)	1	(8)	ns
	B1	0	(0)	2	(100)	ns	1	(33)	1	(50)	ns
	$B2_3$	7	(23)	2	(14)	ns	5	(19)	4	(22)	ns
	D	1	(11)	3	(21)	ns	2	(22)	2	(14)	ns
iucD	A	4	(100)	7	(54)	ns	3	(75)	8	(62)	ns
	B1	2	(67)	1	(50)	ns	1	(33)	2	(100)	ns
	$B2_3$	17	(57)	12	(86)	ns	15	(58)	14	(78)	ns
	D	9	(53)	9	(56)	ns	6	(40)	12	(67)	ns
iroN	A	3	(75)	4	(31)	ns	2	(50)	5	(38)	ns
	B1	2	(67)	1	(50)	ns	1	(33)	2	(100)	ns
	$B2_3$	25	(83)	7	(50)	0,032	21	(81)	11	(61)	ns
	D	7	(41)	5	(36)	ns	5	(33)	7	(39)	ns
irp2	A	4	(100)	5	(42)	ns	4	(100)	5	(42)	ns
	B1	2	(67)	1	(50)	ns	1	(33)	2	(100)	ns
	$B2_3$	27	(90)	14	(100)	ns	24	(92)	17	(94)	ns
	D	11	(65)	10	(63)	ns	11	(73)	10	(56)	ns
kpsMTII	A	2	(50)	0	(100)	0,044	0	(0)	2	(17)	ns
	B1	0	(0)	1	(50)	ns	0	(0)	1	(50)	ns
	$B2_3$	24	(80)	8	(57)	ns	20	(77)	12	(67)	ns
	D	13	(76)	10	(63)	ns	12	(80)	11	(61)	ns
iss	A	4	(100)	2	(17)	0,006	3	(75)	3	(25)	ns
	B1	1	(33)	0	(0)	ns	0	(0)	1	(50)	ns
	$B2_3$	6	(20)	1	(7)	ns	3	(12)	4	(22)	ns
	D	1	(12)	5	(36)	ns	1	(11)	5	(28)	ns

		Prevalence [no. (%)]							
		FQ				SXT			
		S		R	**P**	S		R	P
Virulence gene	Phylogenetic group								
usp	A	1	(25)	0	(0) ns	0	(0)	1 (8)	ns
	B1	0	(0)	0	(0) ns	0	(0)	0 (0)	ns
	$B2_3$	13	(43)	6	(43) ns	11	(42)	8 (44)	ns
	D	2	(25)	0	(0) ns	2	(33)	0 (0)	ns

Virulence traits with ≥10 % prevalence were included.
FQ: Fluoroquinolone; SXT: sulfamethoxazole/trimethoprim; S: Susceptible; R: Resistant;

et al. 2003), 0% (Johnson et al. 2005) and 11% (Moreno et al. 2006) of group B2 fluoroquinolone resistant isolates, while among *E. coli* isolated from 2005 to 2007 the percentage raised upon 50% (Takahashi 2009) and 49% (Johnson et al. 2009). The relatively high percentage (31%) of fluoroquinolone-resistant isolates belonging to group B2 in our collection, comprising *E. coli* isolates from 2004–2007, is therefore in agreement with this trend.

The prevalence of virulence genes among the studied UPEC isolates from Slovenia revealed that, resistant isolates possessed less virulence genes than susceptible isolates and vice versa, which is in accordance with results of similar studies from other countries (Johnson et al. 2003, Johnson et al. 2005, Piatti et al. 2008). However, the examined UPEC collections exhibited distinct significant associations of fluoroquinolone and sulfamethoxazole/trimethoprim resistance pattern with particular virulence genes. For example, in our study we found that, the virulence genes *hra* and *iroN* were statistically significant among fluoroquinolone- susceptible phylogenetic group B isolates, while Piatti et al. (2008) reported a significant association between gene *iss* and fluoroquinolone susceptible group B isolates.

To elucidate the basis of such differences further studies are needed, as for now we can only speculate that sample size, the characteristics of the studied isolates and their hosts might be relevant. In addition, the evolutionary origin of the association between possession of virulence factors and susceptibility needs to be clarified. To this end several hypothesis have been postulated: (i) acquisition/loss of pathogenicity islands, (ii) incompatibility of plasmids encoding virulence and resistance, (iii) less virulent strains are more prone to acquire resistance, (iv) acquisition of resistance promotes loss of virulence.

To summarise, our results in comparison to studies performed before 2004 show a steep increase in the prevalence of fluoroquinolone-resistant strains belonging to the B2 group. It is of great concern that *E. coli* strains of the B2 group, which are known to exhibit the greatest virulence potential, are readily acquiring resistance to fluoroquinolones. These strains addtionally equipped with CTX-M plasmids carrying extended-spectrum beta-lactamases (ESBL) and plasmid-mediated quinolone resistance (PMQR) genes might be the source of highly resistant and virulent clonal groups, such as *E. coli* ST131.

Povzetek

Bakterije vrste *Escherichia coli* so sicer del normalne črevesne mikrobiote ljudi in živali s stalno telesno temperaturo, vendar med njimi obstajajo tudi (potencialno) patogeni sevi, ki lahko povzročijo različne okužbe. Od komenzalnih sevov se običajno ločijo po prisotnosti številnih genov z zapisi za dejavnike virulence, ki bakterijam med drugim omogočajo pritrjanje na gostiteljske celice, poškodbe gostiteljske celice, privzem železa in izogibanje imunskemu sistemu. Uropatogeni sevi *E. coli* so poglavitni povzročitelji nezapletenih okužb sečil. Za zdravljenje teh okužb se najpogosteje uporabljajo protimikrobne snovi sulfametoksazol/trimetoprim in fluorokinoloni vendar je zaradi naraščanja odpornih sevov zdravljenje vse težje. Zato nas je zanimalo ali obstaja kakšna povezava med genetskim ozadjem uropatogenih

sevov in odpornostjo proti sulfametoksazol/trimetoprimu in fluorokinolonom. V našo raziskavo smo vključili 99, za protimikrobni učinkovini občutljivih in odpornih bakterij *E. coli*, ki so bile izolirane iz urina bolnikov z vnetjem sečil. Vse seve smo uvrstili v filogenetske skupine in jih pregledali za prisotnost 21-ih genov z zapisom za dejavnike virulence. Ugotovili smo, da je največ občutljivih izolatov iz filogenetske skupine B2, odporni izolati pa so enakomerno razporejeni v filogenetskih skupinah A, B2 in D in, da imajo izolati iz filogenetske skupini B2 največ genskih zapisov za virulentne dejavnike. Poleg tega smo izsledili nekatere statistično značilne povezave med prisotnostjo genskega zapisa za virulentne dejavnike, z uvrstitvijo seva v filogenetsko skupino in odpornostjo proti sulfametoksazol/trimetoprimu in/ali fluorokinolonom. Zaključki naše raziskave so, da so sevi z večjim naborom genov, ki pripomorejo k virulenci, bolj občuljivi za protimikrobni učinkovini in obratno ter, da so uropatogeni izolati *E. coli* iz Slovenije po svojem naboru genskih zapisov za dejavnike virulence podobni uropatogenim izolatom iz drugih geografskih okolij. Razlika je le v značilnih statističnih povezavah posameznih genov s filogenetskimi skupinami.

Acknowledgements

This research was financed by Grant P1-0198 from the Slovenian Research Agency (ARRS). We also thank the OMM Department of The Institute of Public Health of Slovenia for the identified strains.

References

Car, J., Švab, I., Kersnik, J., Vegnuti, M,. 2003. Management of lower urinary tract infection in women by Slovene GPs. Fam. Pract., 20, 452–6.

Clermont, O., Bonacorsi. S., Bingen, E., 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol., 66, 4555–4558.

Clinical and Laboratory Standards Institute, 2007. Performance standards for antimicrobial susceptibility testing; seventeenth informational supplement. M100-S17. Clinical and Laboratory Standards Institute, Wayne, PA.

Drews, S.J., Poutanen, S.M., Mazzulli, T., McGeer A.J., Sarabia, A., Pong-Porter, S., Rzayev, Y., Willey, B., Green, K., Low, D.E., 2005. Decreased prevalence of virulence factors among ciprofloxacin-resistant uropathogenic *Escherichia coli* isolates. J. Clin. Microbiol., 43, 4218–20.

Horcajada, J.P., Soto, S., Gajewski, A., Smithson, A., Jiménez de Anta, M.T., Mensa, J., Vila, J., Johnson, J.R., 2005. Quinolone-resistant uropathogenic *Escherichia coli* strains from phylogenetic group B2 have fewer virulence factors than their susceptible counterparts. J. Clin. Microbiol.; 43: 2962–4.

Johnson, J.R., Kuskowski, M.A., O'Bryan, T.T., Colodner, R., Raz, R. 2005. Virulence genotype and phylogenetic origin in relation to antibiotic resistance profile among *Escherichia coli* urine sample isolates from Israeli women with acute uncomplicated cystitis. Antimicrob. Agents. Chemother., 49, 26–31.

Johnson, J.R., Kuskowski, M.A., Owens, K., Clabots, C., Singer, R.S., 2009. Virulence genotypes and phylogenetic background of fluoroquinolone-resistant and susceptible *Escherichia coli* urine isolates from dogs with urinary tract infection. Vet. Microbiol., 136, 108–114.

Johnson, J.R., Kuskowski, M.A., Owens, K., Gajewski, A., Winokur, P.L., 2003. Phylogenetic origin and virulence genotype in relation to resistance to fluoroquinolones and/or extended-spectrum cephalosporins and cephamycins among *Escherichia coli* isolates from animals and humans. J. Infect. Dis., 188, 759–68.

Le Bouguenec, C., Archambaud, M., Labigne, A., 1992. Rapid and specific detection of the *pap*, *afa*, and *sfa* adhesin-encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. J. Clin. Microbiol., 30, 1189–1193.

Lindič, J., 2005. Pristop k bolniku z okužbo sečil. [Accession to a patient with urinary tract infection]. In: Fras Z, Poredoš P, eds. 47. Tavčarjevi dnevi. [47th Tavčar's days]. Zbornik prispevkov. [Proceedings]. Ljubljana: Medical Faculty;. p. 137–48.

Moreno, E., Prats, G., Sabaté, M., Pérez, T., Johnson, J., Andreu, A., 2006. Quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole resistance in relation to virulence determinants and phylogenetic background among uropathogenic *Escherichia coli*. J. Antimicrob. Chemother., 57, 204–211.

Oelschlaeger, T.A., Dobrindt, U., Hacker, J., 2002.Virulence factors of uropathogens. Curr. Opin. Urol., 12, 33–8.

Piatti, G., Mannini, A., Balistreri, M., Schito, A.M., 2008. Virulence factors in urinary *Escherichia coli* strains: Phylogenetic background and quinolone and fluoroquinolone resistance. J. Clin. Microbiol., 46, 480–487.

Picard, B., Garcia, J.S., Gouriou, S., Duriez, P., Brahimi, N., Bingen, E., Elion, J., Denamur, E., 1999. The link between phylogeny and virulence in Escherichia coli extraintestinal infection. Infect. Immun., 67, 546–53.

Takahashi, A., Muratani, T., Yasuda, M., Takahashi, S., Monden, K., Ishikawa, K., Ishikawa, K., Kiyota, H., Arakawa, S., Matsumoto, T., Shima, H., Kurazono, H., Yamamoto, S., 2009. Genetic profiles of fluoroquinolone-resistant *Escherichia coli* isolates obtained from patients with cystitis: Phylogeny, virulence factors, paiusp subtypes, and mutation patterns. J. Clin. Microbiol.,47, 791–5.

Vila, J., Simon, K., Ruiz, J., Horcajada, J.P., Velasco, M., Barranco, M., Mensa, J., 2002. Are quinolone-resistant uropathogenic *Escherichia coli* less virulent? J. Infect. Dis., 186, 1039–42.