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Prevalence, distribution and genetic association of adhesin gene sequences of *Escherichia coli* isolates from urinary tract infections in Slovenia

Prevalenca, porazdelitev in genetska asociacija zapisov za adhezine v izolatih bakterije *Escherichia coli* iz okužb sečil v Sloveniji

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Abstract. 110 uropathogenic *Escherichia coli* (UPEC) strains obtained from the Institute of Microbiology and Immunology of the Medical Faculty in Ljubljana, Slovenia were screened with molecular biology methods for the well characterized adhesin gene sequences: *fimH* (Type 1 fimbriae), *papC*, *papGII* and *papGIII* (P-fimbriae), *sfa* (S-fimbriae) and *afa/dra* (Afa/Dr adhesins). The *fimH* gene nucleotide sequences were detected in 97% of the isolates, *papC* in 49%, *papGII* in 34%, *papGIII* in 13%, *sfa/foc* in 24% and *afa/dra* sequences were harbored by 2% of the tested isolates. *FimH* sequences were found with similar prevalence in *E. coli* strains of all four phylogenetic groups A, B1, B2 and D. *papC* sequences were also found in all phylogenetic groups, but they were the most prevalent (64%) in the B2 group. The *papGII* showed the highest prevalence in the D group (48%), but *papGIII* adhesin sequences were exclusively found in the B2 group. A very high prevalence of S-fimbriae in the B2 group was detected. The analysis of co-associations of adhesin gene sequences and some other traits revealed that *papC* gene sequences were co-associated with P-fimbriae adhesin gene sequences *papGII* and *papGIII* and with S-fimbriae *sfa/foc* sequences. A negative association was found between *papGIII* and *traT* and between *papGIII* and RepFIB sequence. Interestingly, a negative association was also visible between integrons and P- and S-fimbriae, albeit the association was not statistically significant.

Key words. uropathogenic *Escherichia coli*, UPEC, adhesin, fimbriae

Izvleček. 110 uropatogenih sevov *Escherichia coli*, ki so jih na Inštitutu za mikrobiologijo in imunologijo Medicinske fakultete v Ljubljani osamili iz diagnostičnih vzorcev urina, smo z molekularnobiološkimi metodami analizirali z namenom določiti gene, ki imajo zapise za adhezine: *fimH* (fimbrije tipa 1), *papC*, *papGII* in *papGIII* (fimbrije P), *sfa* (fimbrije S) in *afa/dra* (adhezini Afa/Dr). Nukleotidno zaporedje gena *fimH* smo odkrili v 97 % izolatov, *papC* v 49 %, *papGII* v 34 %, *papGIII* v 13 %, *sfa/foc* v 24% in zaporedja *afa/dra* smo našli v 2 % vseh preučevanih izolatih. Prevalenca zaporedja *fimH* po posameznih filogenetskih skupinah A, B1, B2 in D je primerljiva. V vseh štirih filogenetskih skupinah smo našli tudi zaporedja *papC*, največja prevalenca je bila v skupini B2, kjer je kar 64 % izolatov vsebovalo to zaporedje. Zaporedje *papGII* je imelo največjo prevalenco v skupini D. Zaporedje *papGIII* smo našli izključno v skupini B2. V skupini B2 smo odkrili tudi veliko prevalenco fimbrij S (45 %). Analiza asociacij zapisov za adhezine z drugimi zapisi je pokazala, da je zaporedje *papC* asociirano z zaporedji *papGII* in *papGIII* ter zaporedji *sfa/foc*. Negativno povezavo smo našli med *papGIII* in *traT* ter med *papGIII* in zaporedji RepFIB. Med integroni in fimbrijami P in S smo tudi našli negativno povezavo, a le ta ni bila statistično značilna.

Ključne besede. uropatogena *Escherichia coli*, UPEC, adhezin, fimbrija

Introduction

Escherichia coli (*E. coli*) is a very diverse bacterial species found naturally in the intestinal tract of all humans and many other animal species. Even though *E. coli* is known to be part of the normal gut flora, some strains – that are pathogenic – cause a wide variety of different intestinal and extraintestinal diseases (Marrs & al. 2005). Typical extraintestinal infections due to *E. coli* include urinary tract infections (UTI) (Russo & Johnson 2006).

Any component of a microbe that is required for, or potentiates its ability to cause disease is called a virulence factor. The best known virulence factors are adhesins, toxins, polysaccharide coatings, invasins and iron uptake systems. Among the first virulence factors that come into play during establishment of an infection are adhesins. Besides their primary role as adhesin molecules, they can also function as invasins, promoters of biofilm formation and transmitors of signals to epithelial cells resulting in inflammation.

Type 1 fimbriae are the most common adhesive organelles of *E. coli* strains. They are encoded by the vast majority of uropathogenic *E. coli* (UPEC) isolates and many other pathogenic and commensal isolates (Bower & al. 2005). They are also found in other bacteria, such as *Salmonella typhimurium*, *Pseudomonas putida* and *Klebsiella pneumoniae* (Capitani & al. 2006). Receptors for type 1 fimbriae are present on erythrocytes, buccal epithelial cells, intestinal cells, vaginal cells and uroepithelial cells (Johnson 1991). The *fimH* gene encodes the minor subunit protein FimH that mediates binding to the receptor. FimH has several variants: UPEC strains have a FimH that binds both monomannose and trimannose containing gycoprotein receptors, while commensal *E. coli* isolates typically show high affinity binding to only trimannose residues (Bower & al. 2005). Type 1 fimbriae function not just as adhesins, but also as invasins for bladder epithelial cells (Martinez & al. 2000).

P fimbriae are among the best studied fimbrial adhesive fibres of UPEC strains. The P fimbrial adhesin molecule (PapG) recognizes globoseries of glycolipids as receptors (Zhang & Foxman 2003). The *papC* gene encodes the outer membrane usher protein that is required for ordered P fimbriae assembly (Thanassi & al. 1998). Many studies showed that P fimbriae occur more frequently among UPEC than fecal isolates. It was estimated, that about 80% of *E. coli* isolates from patients with pyelonephritis possess P-fimbriae. Based on binding specificities, P fimbriae are grouped into three major classes (I, II and III); class II (*papGII*) is more often found in pyelonephritic strains and class III (*papGIII*) in cystitis strains (Zhang & Foxman 2003).

S fimbriae bind to sialyl galactosides. Studies showed that *E. coli* UTI isolates were at least two times more likely to carry S fimbriae genes (*sfa* operon) than fecal strains (Zhang & Foxman 2003).

The 13 known adhesins of the Afa/Dr family all bind to the Dr^a blood group antigen present on the complement regulatory molecule CD55, also known as decay-accelerating factor (DAF) (Bower & al. 2005). The *E. coli* strains harboring these adhesins have been found to be associated with UTIs and also with various enteric infections (Servin 2005).

The subunit proteins of adhesins are seriously considered as possible vaccines against *E. coli* infections (Oelschlaeger & al. 2002). Since UTIs and other extraintestinal infections due to *E. coli* cause considerable costs to the health system vaccines against *E. coli* are searched for (Russo & Johnson 2006). To evaluate the potential of different adhesins as vaccines it is necessary to investigate the presence of individual adhesins among pathogenic strains. To our knowledge, no such data are available for Slovenian uropathogenic *E. coli* (UPEC) strains. We therefore, analyzed a collection of 110 UPEC strains, that were previously screened for antibiotic resistance and horizontal gene transfer elements (Rijavec & al. 2006), for the presence of the following adhesin gene sequences: *fimH* (Type 1 fimbriae), *papC*, *papGII* and *papGIII* (P fimbriae), *sfa* (S-fimbriae) and *afa/dra* (afimbrial adhesin).

Table 1: Oligonucleotide primers and PCR conditions to detect adhesin genes
Tabela 1: Oligonukleotidni začetniki in pogoji PCR za ugotavljanje genskih zapisov za adehezine

Gene	Oligonucleotide sequence (5' to 3')	Size of product (bp)	PCR conditions			Reference
fimH	tgcagaacggataagccgtgg gcagtcacctgccctccggta	508	95°C 94°C 60°C 72°C 72°C	2,5 min 0,5 min 0,5 min 1 min 10 min	1× 30× 1×	Johnson & Stell, 2000
papC	gacggctgtactgcagggtgtggcg atatccttttctgcagggatgcaata	328	94°C 94°C 65°C 72°C 72°C	3 min 2 min 1 min 2 min 10 min	1× 25× 1×	Le Bouguenec & al., 1992
papGII	gggatgagcgggcctttgat cgggccccaagtaactcg	190	95°C 94°C 55°C 72°C 72°C	2,5 min 0,5 min 1 min 0,5 min 7 min	1× 25× 1×	Johnson & Brown, 1996
papGIII	ggcctgcaatggatttacctgg ccaccaaatgaccatgccagac	258	94°C 94°C 63°C 72°C 72°C	2,5 min 0,5 min 0,5 min 3 min 10 min	1× 25× 1×	Johnson & Brown, 1996
sfa/foc	ctccggagaactgggtgcatcttac cggaggagtaattacaaacctggca	410	94°C 94°C 65°C 72°C 72°C	3 min 2 min 1 min 2 min 10 min	1× 25× 1×	Le Bouguenec & al., 1992
afa/dra	gctgggcagcaaactgataactctc catcaagctgtttgttcgtccgccg	750	94°C 94°C 65°C 72°C 72°C	3 min 2 min 1 min 2 min 10 min	1× 25× 1×	Le Bouguenec & al., 1992

Material and methods

Bacterial strains and media

A total of 110 *E. coli* isolates (DL strains) from humans with urinary tract infections collected in 2002, at the Institute of Microbiology and Immunology, Medical Faculty, Ljubljana, Slovenia were studied. Only one isolate from each patient was analyzed. Ninety-four (86%) of the patients were women. The strains had already been examined for prevalence of antibiotic resistances further, the serotype and phylogenetic groups were assigned and traits typical of horizontal gene transfer (*traT*, integrons, *rep*) were searched for (Rijavec & al. 2006). For cultivation of strains Luria Bertani medium or agar were used.

Detection of adhesin genes

The primers and PCR conditions used to amplify adhesin genes with polymerase chain reaction (PCR) are listed in Table 1. DNA to be amplified was released from whole organisms by boiling according to Le Bouguenec et al. (Le Bouguenec & al. 1992). Amplification was performed in an automated thermal cycler (UNOII, Biometra, Göttingen, Germany) in a 50 µl reaction mixture containing template DNA (10 µl of boiled lysate), 20 pmol of forward and reverse primer, 0,2 mM of dNTP mixture, 1,25 U *Taq* DNA polymerase and 2,5 mM $MgCl_2$ in 1× PCR buffer (Fermentas, Vilnius, Lithuania).

Dot blot hybridization experiments using the DIG DNA labelling and detection kit (Roche, Mannheim, Germany) were performed to validate the PCR assays. Probes were prepared using the same primers as for the PCR experiments and labelled with digoxigenin. The template DNA samples were the same as in the PCR experiments.

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.matforsk.no/ola/fisher.htm and the level of significance was set at a P value < 0.05.

Results

Prevalence of adhesin genes

The presence of adhesin genes in the genomes of DL strains was screened by PCR and validated in the hybridization experiments. Figure 1 gives an example for *papGIII* detection. Among the tested adhesive organelles, the type 1 fimbriae were the most prevalent – the *fimH* gene nucleotide sequences were detected in 107 strains (97%). The P-fimbriae were also abundant, in 54 strains (49%) *papC* encoding gene sequence was found, 37 strains (34%) harbored the class II *papG* adhesin sequence and 14 strains (13%) harbored the class III *papG* adhesin. 26 (24%) possessed the S fimbriae typical gene sequence *sfa/foc*. Only 2 strains (2%) harbored *afa/dra* sequences (Figure 2).

Distribution of adhesin genes among phylogenetic groups

E. coli isolates can be divided into four main phylogenetic groups A, B1, B2 and D (Herzer & al. 1990). Analysis of the distribution of adhesin gene sequences among the previously determined phylogenetic groups of DL strains (Rijavec & al. 2006) revealed that different adhesin gene sequences were differently distributed (Table 2). *FimH* sequences were found with similar prevalence in strains of all four phylogenetic groups, *papC* sequences were found in all phylogenetic groups, but they were most prevalent (64%) among B2 group strains. The association of *papC* with the B2 group was statistically significant. The distribution of the P-fimbriae adhesins *papGII* and *papGIII*, however, differed. *papGII* sequences showed the highest prevalence in the D group (48%), albeit the association was not statistically significant. In contrast, *papGIII* adhesin sequences were exclusively found among strains of the B2 group. Further, a very high, statistically significant, prevalence of S-fimbriae in the B2 group was detected.

Co-associations of adhesin genes

The analysis of co-associations of adhesin gene sequences and some other traits revealed (Table 3) that the P-fimbriae usher *papC* gene sequences were 100% co-associated with P-fimbriae adhesin

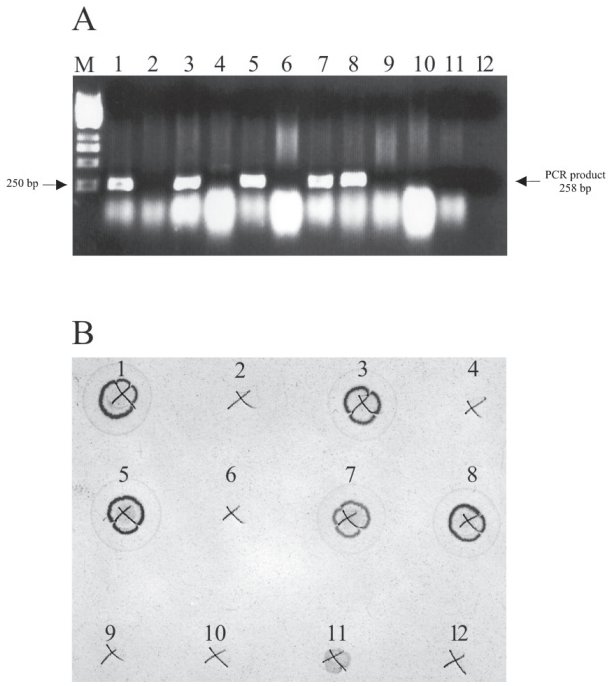

Figure 1: An example of detection of adhesin genes – detection of the *papGIII* gene

(A) Visualization of PCR products obtained in PCR reactions on lysates of DL strains with primers specific for the *papGIII* gene (1% agarose gel, stained with ethidium bromide).

M: marker – 1 kb DNA ladder (Fermentas, Vilnius, Lithuania); 1: strain DL1 (*papGIII*+); 2: strain DL9 (*papGIII*-); 3: strain DL12 (*papGIII*+); 4: strain DL23 (*papGIII*-); 5: strain DL30 (*papGIII*+); 6: strain DL49 (*papGIII*-); 7: strain DL59 (*papGIII*+); 8: strain DL62 (*papGIII*+); 9: strain DL89 (*papGIII*-); 10: strain DL90 (*papGIII*-); 11: laboratory strain DH5α (*papGIII*-) and 12: negative control – a PCR reaction with sterile water instead of a lysate.

(B) Validation of the PCR assay with DIG hybridization of a *papGIII* specific probe on *papGIII* PCR products (10 µl) bound to a nylone membrane.

1: strain DL1 (*papGIII*+); 2: strain DL9 (*papGIII*-); 3: strain DL12 (*papGIII*+); 4: strain DL23 (*papGIII*-); 5: strain DL30 (*papGIII*+); 6: strain DL 49 (*papGIII*-); 7: strain DL59 (*papGIII*+); 8: strain DL62 (*papGIII*+); 9: strain DL89 (*papGIII*-); 10: strain DL90 (*papGIII*-); 11: laboratory strain DH5α (*papGIII*-) and 12: negative control – a PCR reaction with sterile water instead of a lysate.

Slika 1: Primer detekcije genskega zapisa za adhezin – detekcija gena *papGIII*

(A) Vizualizacija produktov PCR dobljenih v reakcijah PCR na lizatih sevov DL z začetnimi oligonukleotidi specifičnimi za gen *papGIII* (1 % agarozni gel, obarvan z etidijevim bromidom).

M: standard – 1 kb DNA-lestvica (Fermentas, Vilnius, Litva); 1: sev DL1 (*papGIII*+); 2: sev DL9 (*papGIII*-); 3: sev DL12 (*papGIII*+); 4: sev DL23 (*papGIII*-); 5: sev DL30 (*papGIII*+); 6: sev DL49 (*papGIII*-); 7: sev DL59 (*papGIII*+); 8: sev DL62 (*papGIII*+); 9: sev DL89 (*papGIII*-); 10: sev DL90 (*papGIII*-); 11: laboratorijski sev DH5α (*papGIII*-) in 12: negativna kontrola – reakcija PCR s sterilno vodo namesto lizata.

(B) Preverjanje PCR z DIG-hibridizacijo z vezavo sonde specifične za *papGIII* na produkte *papGIII* iz PCR (10 µl) vezane na nylonski membrani.

1: sev DL1 (*papGIII*+); 2: sev DL9 (*papGIII*-); 3: sev DL12 (*papGIII*+); 4: sev DL23 (*papGIII*-); 5: sev DL30 (*papGIII*+); 6: sev DL 49 (*papGIII*-); 7: sev DL59 (*papGIII*+); 8: sev DL62 (*papGIII*+); 9: sev DL89 (*papGIII*-); 10: sev DL90 (*papGIII*-); 11: laboratorijski sev DH5α (*papGIII*-) in 12: negativna kontrola – reakcija PCR s sterilno vodo namesto lizata.

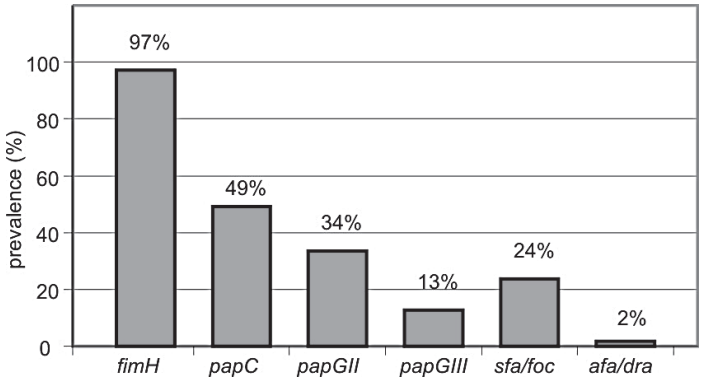

Figure 2: Prevalence (in % of the total 110 DL strains) of adhesin gene sequences
Slika 2: Prevalenca (v % od 110 preučevanih sevov E. coli) genskih zapisov za adhezine

gene sequences *papGII* and *papGIII*. Further, *papC* gene sequences were also statistically significantly co-associated with S-fimbriae *sfa/foc* sequence. The P-fimbriae adhesin gene sequences *papGIII*, but not *papGII*, were statistically significantly co-associated with S-fimbriae *sfa/foc* sequence. A statistically significant negative association was found between *papGIII* and *traT* and between *papGIII* and RepFIB sequences. Interestingly, a negative association was also visible between integrons and P- and S-fimbriae, albeit the association was not statistically significant.

Co-associations of adhesin genes (Table 3) *fimH* and *afa/dra* were not analyzed, due to either very high or low prevalence, respectively.

Discussion

In the presented study 110 UTI *E. coli* strains isolated in Ljubljana, Slovenia, were characterized using PCR with primers specific for adhesin genes: *fimH*, *papC*, *papGII*, *papGIII*, *sfa/foc* and *afa/dra*.

Among the tested adhesin gene sequences the prevalence of *fimH* gene sequences was the highest, almost 100%. The high prevalence of *fimH* sequences found in our study assures a good possibility

Table 2: Distribution of adhesin gene sequences among *E. coli* phylogenetic groups
Tabela 2: Razporeditev genskih zapisov za adhezine po filogenetskih skupinah *E. coli*

	Prevalence of trait (no. [%] of isolates) within phylogenetic group[a]			
Toxin gene	A (n = 28)	B1 (n = 6)	B2 (n = 55)	D (n = 21)
fimH	28 (100)	5 (83)	53 (96)	21 (100)
papC	8 (29)$^{(*)}$	1 (17)	35 (64)**	10 (48)
papGII	5 (18)	1 (17)	21 (38)	10 (48)
papGIII	0 (0)$^{(*)}$	0 (0)	14 (25)***	0 (0)
sfa/foc	1 (4)$^{(**)}$	0 (0)	25 (45)***	0 (0)$^{(**)}$
afa/dra	1 (4)	0 (0)	1 (2)	0 (0)

[a]*P* values (Fisher's exact test) are indicated by asterisks where *P* is <0,05. Symbols: *, *P*<0,05; **, *P*≤0,01; ***, *P*≤0,001. Parentheses indicate negative associations.

[a] vrednost *P* (Fisherjev eksaktni test) <0,05 je nakazana z zvezdicami: simboli *, *P*<0,05; **, *P*≤0,01; ***, *P*≤0,001. Negativne povezave so označene z oklepajem.

Table 3: Co-association of tested adhesin genes and some other traits
Tabela 3: Vezanost genskih zapisov za adhezine in nekaterih drugih lastnosti

	Prevalence of trait (no. [%] of isolates)[a]																		
	C+		*C-*		*P*	*GII+*		*GII-*		*P*	*GIII+*		*GIII-*		*P*	*sfa/foc+*		*sfa/foc-*		*P*
Trait	n = 54		n = 56			n = 37		n = 73			n = 14		n = 96			n = 26		n = 84		
PapC																				
papGIII	37	(69)	0	(0)	<0,001															
papGIII	14	(26)	0	(0)	<0,001	3	(8)	11	(15)	NS[b]										
sfa/foc	23	(43)	3	(5)	<0,001	12	(32)	14	(19)	NS	11	(79)	15	(16)	<0,001					
TraT	26	(48)	37	(66)	0,082-NS	20	(54)	43	(59)	NS	4	(29)	59	(61)	0,040	11	(42)	52	(62)	NS
integron	14	(26)	20	(36)	NS	7	(19)	27	(37)	0,080-NS	2	(14)	32	(33)	NS	4	(15)	30	(36)	0,060-NS
RepFIA	12	(22)	8	(14)	NS	7	(19)	13	(18)	NS	2	(14)	18	(19)	NS	6	(23)	14	(17)	NS
RepFIB	27	(50)	30	(54)	NS	21	(57)	36	(49)	NS	3	(21)	54	(56)	0,020	10	(38)	47	(56)	NS
RepFIIA	13	(24)	11	(20)	NS	9	(24)	15	(21)	NS	1	(7)	23	(24)	NS	4	(15)	20	(24)	NS

[a] In the table are not included *fimH* and *afa/dra* due to their too high or too low prevalence, 97% and 2%, respectively. Included are data for *traT*, integrons and replication regions RepFIA, RepFIB, RepFIIA (Rijavec & al. 2006).
[b] NS – not statistically significant

[a] V tabelo niso vključeni *fimH* in *afa/dra*, ker imata previsoko oz. prenizko prevalence (*fimH* = 97% in *afa/dra* = 2%). Vključeni so podatki za *traT*, integrone in replikacijske regije RepFIA, RepFIB, RepFIIA (Rijavec & al. 2006).
[b] NS – ni statistično značilno

Table 4: Comparison of results from different studies of UTI adhesin genes
Tabela 4: Primerjava rezultatov različnih raziskav genskih zapisov za adhezine

	Adhesin gene prevalence (%)						
Study	*fimH*	*papC/A*	*papGII*	*papGIII*	*sfa/foc*	*afa/dra*	Ref.
76 pyelonephritis strains (Japan)	na	78	na	na	42	12	Kanamaru & al. 2003, Yamamoto & al. 2001
74 cystitis strains (USA)	na	35	5	31	36	4	Johnson & al. 2001
170 pyelonephritis strains (USA)	99	68	60	9	na	17	Johnson & al. 2005b
194 cystitis strains (Japan)	na	64	na	na	37	9	Kanamaru & al. 2003, Yamamoto & al. 2001
100 cystitis strains (Israel)	na	46	31	17	37	14	Johnson & al. 2005a
78 UTI strains (Romunia)	86	36	na	na	23	14	Usein & al. 2001
110 UTI strains (Slovenia)	97	49	34	13	24	2	this study

for prevention of infection with the vaccine against the type 1 fimbriae that is already in phase II/III trial in theUS (Russo & Johnson 2006).

The P-fimbriae *papC* sequences were found in 49% of the tested DL strains. Comparison of our data with data on prevalences of *papC* or *papA* (encoding major fimbrial subunit PapA) from other studies (Table 4) showed a similar prevalence among UTI strains from Romania and cystitis strains from Israel and USA, but higher prevalence of *papC/A* among the pyelonephritis strains from USA and cystitis and pyelonephritis strains from Japan, compared to the prevalence in DL strains investigated in this study.

The *papC* sequences were 100% co-associated with the adhesin genes *papGII* and *papGIII*. This was expected, since *papGII* and *papGIII* are alleles of P-fimbriae adhesins and each strain harboring either *papGII* or *papGIII* sequences also harbors *papC* sequences.

Interestingly, in our study, as well as, in the study of Johnson *et al*. (Johnson & al. 2005b), *papGII* exhibited the highest prevalence among strains of the D group. This is in contrast to the results of *papC* and *papGIII* for which the highest prevalence was found among strains belonging to the B2 group, which is known to exhibit the highest prevalence of virulence traits.

Further, *papC* sequences were also strongly co-associated with *sfa/foc* sequences. This is surprising, as to our knowledge no previous study reported such a correlation. Co-association of virulence factors are expected, when they are physically joined, and it is well known that uropathogenic strains carry large chromosomal regions, termed pathogenicity islands (PAI) that encode several virulence factors. A number of PAIs have been identified in uropathogenic strains (Oelschlaeger & al. 2002), however to our knowledge no PAI harboring S-fimbriae and P-fimbriae has ever been described.

A negative association of P and S fimbriae with integrons is, even though not statistically significant, evident. Integrons are known to carry resistance genes for different/multiple antibiotics (Mazel 2006). Further, it is well known, that antibiotic-sensitive isolates possess more virulence factors than antibiotic-resistant isolates (Johnson & al. 2003, Starčič Erjavec & al. 2007). Therefore, it is reasonable that in strains with virulence associated adhesins, the prevalence of integrons is smaller.

It is worth to be noted, that not all studies (Table 4) support the assumption, that the *papGIII* allele is associated with cystitis isolates and *papGII* with pyelonephritis (Zhang & Foxman 2003). For example the study on Israelian cystitis isolates showed, that cystitis isolates have a higher prevalence of the *papGII* allele than the *papGIII* allele.

Further studies on different cystitis/pyelonephritis isolates are needed to clarify the importance of different *papG* alleles and to clarify the basis of the correlation between P and S fimbriae.

Conclusions

To summarise and conclude:

1. 110 uropathogenic *Escherichia coli* (UPEC) strains were screened with molecular biology methods for the well characterized adhesin gene sequences: *fimH* (Type 1 fimbriae), *papC*, *papGII* and *papGIII* (P-fimbriae), *sfa* (S-fimbriae) and *afa/dra* (Afa/Dr adhesins);
2. the prevalence, the distribution and the genetic associations of the tested adhesin gene sequences were determined;
3. the *fimH* gene nucleotide sequences were detected in 97% of the isolates, *papC* in 49%, *papGII* in 34%, *papGIII* in 13%, *sfa/foc* in 24% and *afa/dra* sequences were harbored by 2% of the tested isolates;
4. *fimH*, *papC*, *papGII* were found in all four *E. coli* phylogenetic groups, *sfa/foc* and *afa/dra* in A and B2 group and the *papGIII* was found only in the B2 group;
5. *papC* gene sequences were co-associated with P-fimbriae adhesin gene sequences *papGII* and *papGIII* and with S-fimbriae *sfa/foc* sequence.

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Povzetek

Bakterija *Escherichia coli* (*E. coli*) je del normalne flore prebavila človeka in toplokrvnih živali. A obstajajo sevi *E. coli*, ki imajo virulentne dejavnike (toksine, adhezine, kapsule, ...) in lahko povzročijo okužbe (driska, vnetje sečil, pljučnica, vnetje možganskih ovojnic, okužbe ran, ...). Okužba sečil je ena izmed najpogostejših bakterijskih infekcij in *E. coli* povzroča veliko večino teh okužb. Zaradi pogostnosti pojavljanja teh okužb so virulentni dejavniki sevov *E. coli*, ki povzročajo te okužbe (UPEC – uropatogena *E. coli*) za preučevanje zelo zanimivi. Kar nekaj adhezinov in fimbrij (fimbrije tipa 1, P-fimbrije, S-fimbrije, Afa/Dr-adheizini) povezujejo s patogenimi sevi UPEC. 110 uropatogenih sevov *Escherichia coli*, ki so jih na Inštitutu za mikrobiologijo in imunologijo Medicinske fakultete v Ljubljani osamili iz diagnostičnih vzorcev urina, smo z molekularnobiološkimi metodami analizirali z namenom določiti gene, ki imajo zapise za adhezine: *fimH* (fimbrije tipa 1), *papC*, *papGII* in *papGIII* (fimbrije P), *sfa* (fimbrije S) in *afa/dra* (adhezini Afa/Dr). Nukleotidno zaporedje gena *fimH* smo odkrili v 97 % izolatov, *papC* v 49 %, *papGII* v 34 %, *papGIII* v 13%, *sfa/foc* v 24% in zaporedja *afa/dra* smo našli v 2 % vseh preučevanih izolatih. Prevalenca zaporedja *fimH* po posameznih filogenetskih skupinah A, B1, B2 in D je primerljiva – je več kot 80 %. V vseh štirih filogenetskih skupinah smo našli tudi zaporedja *papC*, največ v skupini B2 (64 % izolatov). Zaporedje *papGII* je imelo največjo prevalenco v skupini D (48 %). Zaporedje *papGIII* smo našli izključno v skupini B2 (25 %). V skupini B2 smo odkrili tudi veliko prevalenco fimbrij S (45 %). Analiza asociacij zapisov za adhezine z drugimi zapisi je pokazala, da je zaporedje *papC* asociirano z zaporedji *papGII* in *papGIII* ter zaporedji *sfa/foc*. Negativno povezavo smo našli med *papGIII* in *traT* ter med *papGIII* in zaporedji RepFIB. Med integroni in fimbrijami P in S smo tudi našli negativno povezavo, a le ta ni bila statistično značilna. Zbrane informacije o pogostnosti zapisov za adhezine bi lahko bile osnova za načrtovanje cepiv proti patogenim sevom *E. coli*.

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