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Pathogenicity islands, plasmids and iron uptake systems in extraintestinal pathogenic *Escherichia coli* strains

Otoki patogenosti, plazmidi in sistemi za privzem železa v zunajčrevesnih patogenih sevih bakterije *Escherichia coli*

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Abstract: The aim of the presented study was to estimate the prevalence, distribution and associations of different pathogenicity islands (PAI I_{536} to PAI IV_{536}, PAI I_{J96}, PAI II_{J96}, PAI I_{CFT073} and PAI II_{CFT073}), iron uptake systems (genes *iutA*, *iucD*, *iroN*, *iroCD*, *fyuA*, *irp2*, *iha*, *ireA*, and *hbp*) and plasmids among extraintestinal pathogenic *Escherichia coli* (ExPEC) strains isolated from Slovenian patients. Twenty-nine ExPEC isolates obtained from the Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana were investigated for the presence of different pathogenicity islands and iron uptake systems with PCR, the plasmid content of the investigated strains was determined by molecular biology techniques. The significance of the found associations of the studied PAIs and iron uptake systems was analyzed with the Fisher's exact test. PAI IV_{536} was found in 19, PAI II_{CFT073} in 6, PAI I_{CFT073} in 4, and PAI II_{J96} in one of the studied isolates. PAI I_{536}, PAI II_{536}, PAI III_{536} and PAI I_{J96} were not detected in any studied isolate. In 19 of the studied isolates plasmids were detected. The *irp2* was found in 20, *fyuA* in 19, *iucD* and *iutA* in 12, *iha* in 9, *iroN* in 8, *iroCD* in 7, *ireA* in 7 and *hbp* in 4 of studied isolates. PAI IV_{536} was statistically significantly associated with the yersiniabactin siderophore system and PAI I_{CFT073} was statistically significantly associated with the aerobactin siderophore system as well as Iha. To our knowledge this is the first report on PAIs and iron uptake systems among Slovenian ExPEC isolates, as well as a first report on PAIs, iron uptake systems and plasmids among isolates from skin and soft tissue infections.

Key words: extraintestinal pathogenic *Escherichia coli*, ExPEC, pathogenicity island, PAI, plasmid, iron uptake

Izvleček: Cilj raziskave je bil oceniti prevalenco, razporeditev in asociacije različnih otokov patogenosti (PAI I_{536} do PAI IV_{536}, PAI I_{J96}, PAI II_{J96}, PAI I_{CFT073} in PAI II_{CFT073}), sistemov za privzem železa (geni *iutA*, *iucD*, *iroN*, *iroCD*, *fyuA*, *irp2*, *iha*, *ireA*, in *hbp*) in plazmidov v zunajčrevesnih patogenih sevih bakterije *Escherichia coli* (ExPEC) izoliranih iz slovenskih bolnikov. Devetindvajset izolatov ExPEC, ki so jih osamili na Inštitutu za mikrobiologijo in imunologijo Medicinske fakultete v Ljubljani, smo s pomočjo PCR preiskali za prisotnost različnih otokov patogenosti in sistemov za privzem železa, z molekulskobiološkimi tehnikami smo preverjali prisotnost plazmidov v preučevanih sevih. Statistično značilnost povezave preučevanih PAI in sistemov za privzem železa smo ugotavljali s Fisherjevim eksaktnim testom. PAI IV_{536} smo našli v 19, PAI II_{CFT073} v 6, PAI I_{CFT073} v 4 , PAI II_{J96} v enem od preučevanih izolatov. PAI I_{536}, PAI II_{536}, PAI III_{536} in PAI I_{J96} nismo odkrili v nobenem izmed preučevanih izolatov. V 19 sevih smo odkrili plazmide. Gen *irp2* smo našli v 20, *fyuA* v 19, *iucD* in *iutA* v 12, *iha* v 9, *iroN* v 8, *iroCD* v 7, *ireA* v 7 in *hbp* v 4 od preučevanih sevov. PAI IV_{536} je bil statistično značilno povezan s siderofornim sistemom jersiniabaktin in PAI I_{CFT073} je bil statistično značilno povezan s siderofornim sistemom aerobaktin in Iha. Kolikor nam je poznano, je to prva raziskava o PAI in sistemih za privzem železa na zbirki sevov ExPEC iz slovenskih bolnikov, in ki podaja podatke o PAI, sistemih za privzem železa in plazmidih izolatov ExPEC iz infekcij kože in podkožja.

Ključne besede: Zunajčrevesna patogena *Escherichia coli*, ExPEC, otoki patogenosti, PAI, plazmid, sistemi za privzem železa

Introduction

Escherichia coli (*E. coli*) is the most abundant facultative anaerobe of the human intestinal microflora. Despite the fact that it is a commensal bacterium, some *E. coli* strains have acquired specific virulence attributes that confer an increased ability to adapt to new niches and allow them to cause a broad spectrum of disease at either intestinal or extraintestinal sites (Kaper & al. 2004). Often the virulence attributes are genetically linked – located in a subgroup of genomic islands, in the so called pathogenicity islands (PAI) (Schmidt & Hensel 2004). Typical characteristics of PAIs (Tab. 1), apart from encoding virulence genes, are: presence in pathogenic strains but absence or rareness in nonpathogenic strains of the same or related species; size ranging from 10 kb up to 200 kb; relative instability, different G+C content than the core genome, association with tRNA genes, presence of mobile genetic elements (insertion sequences, transposons, integrases, and bacteriophage DNA), flanked by direct repeat sequences and due to different episodes of horizontal gene transfer a mosaic-like structure (Hacker & Kaper 2000, Schmidt & Hensel 2004).

In addition to PAIs, plasmids, extrachromosomal DNA elements that range in size from approximately 300 bp to 2400 kbp, can carry genes encoding virulence factors (Kado 1998). Based on the overall genetic content, two types of plasmids are distinguished. One, designated as non-conjugative or non-transmissible, harbour genes for the initiation and regulation of its replication but do not possess genes required for conjugal transfer. The second type are conjugative or self-transmissible that also carry genes involved in conjugation (Helinski & al. 1996).

Iron is an essential cofactor for many basic metabolic pathways and bacteria have developed specialized iron uptake systems to capture iron. The most prominent are the siderophores, iron-binding molecules that are taken up by special siderophore receptors and ATP-consuming porin-

Table 1: Characteristics of studied PAIs
Tabela 1: Značilnosti preučevanih PAI

PAI name	Size (kbp)	Insertion position	Identified carried virulence (associated) factors	Ref.
PAI I_{536}	76,8	*selC*	alpha-hemolysin, F17-like fimbriae, and CS12-like fimbriae	(Schmidt & Hensel 2004)
PAI II_{536}	102,2	*leuX*	P-related fimbriae, alpha-hemolysin, Hek adhesin, hemagglutinin-like adhesins	(Schmidt & Hensel 2004)
PAI III_{536}	68,1	*thrW*	S-fimbriae, *iro* siderophore system, a HmuR-like heme receptor, a Sap adhesin, a TSH-like hemoglobin protease	(Schmidt & Hensel 2004)
PAI IV_{536} = HPI	30,2	*asnT*	yersiniabactin siderophore system	(Schmidt & Hensel 2004)
PAI I_{J96}	> 170	*pheV*	alpha-hemolysin, P- fimbriae	(Schmidt & Hensel 2004)
PAI II_{J96}	110	*pheU*	alpha-hemolysin, Prs- fimbriae, cytotoxic necrotizing factor I	(Schmidt & Hensel 2004)
PAI I_{CFT073} = PAI-CFT073-*pheV*	123	*pheV*	alpha-hemolysin, P-fimbriae, Iha, autotransporter Sat, aerobactin siderophore system, antigen 43 precursor, capsule gene *kpsTM*	(Lloyd & al. 2007)
PAI II_{CFT073} = PAI-CFT073-*pheU*	52	*pheU*	P-fimbriae	(Lloyd & al. 2007)

like transporters in the bacterial outer membrane (Schaible & Kaufmann 2004). Siderophores can be classified into three groups: (i) the catecholate type (enterobactin, salmochelin = enterochelin), (ii) hydroxamate type (aerobactin) and (iii) a mixed type – a combination of both (yersiniabactin) (Grass 2006, Schaible & Kaufmann 2004). In addition to siderophore synthesis strains can use siderophores produced and released into the extracellular medium by other bacteria and even fungi. In the host, bacteria may use iron sources such as heme, hemoglobin, hemopexin, and iron bound to transferrin and lactoferrin (Braun & Braun 2002). Apart from the siderophores and their receptors, autotransporters, virulence-associated proteins in gram-negative bacteria, can also play a role in obtaining iron for example, the hemoglobin protease Hbp (Otto & al. 2002). All autotransporter proteins are energy-independent secreted via a type 5 secretion system and possess an overall unifying structure, comprising (i) an amino-terminal leader peptide (for secretion across the inner membrane), (ii) the secreted mature protein (or passenger domain), and (iii) a dedicated C-terminal domain, which forms a pore in the outer membrane through which the passenger domain passes to the cell surface (Henderson & Nataro 2001). Hbp, after it is autotransported out of the bacterial cell, interacts specifically with human hemoglobin, degrades it, and subsequently binds the released heme (Otto & al. 1998).

E. coli isolates capable of causing disease outside the intestinal tract, e. g., uropathogenic *E. coli* (UPEC), sepsis-associated *E. coli*, and neonatal meningitis-associated *E. coli*, are classified as extraintestinal pathogenic *E. coli* (ExPEC) (Russo & al. 2000). Within the human intestinal tract, ExPEC may colonize without causing disease, but when they disseminate to other body sites, they elicit, due to encoded virulence factors, pathogenesis (Wiles & al. 2008). In ExPEC isolates many pathogenicity islands were found, among the best known and studied are pathogenicity islands PAI I_{536} to PAI IV_{536} from the uropathogenic *E. coli* strain 536, PAI I_{J96} and PAI II_{J96} from the uropathogenic *E. coli* strain J96 and PAI I_{CFT073} and PAI II_{CFT073} from uropathogenic *E. coli* strain CFT073 (Schmidt & Hensel 2004). Further, among ExPEC strains many iron uptake systems were found, characterized and associated with pathogenesis, among them aerobactin, salmochelin, yersiniabactin, Iha, IreA and Hbp. The aim of the present study was to characterize 29 ExPEC strains isolated from Slovenian patients suffering from extraintestinal *E. coli* infections for the presence of the best characterised PAIs and iron uptake systems. In addition, we aimed to estimate the prevalence of PAIs, iron uptake systems and plasmids, and to analyse the distribution and associations of PAIs and iron uptake systems among ExPEC strains isolated in Slovenia.

Material and methods

Bacterial strains and media

Twenty-nine randomly collected *E. coli* isolates from humans with extraintestinal infections isolated at the Institute of Microbiology and Immunology, Medical Faculty, Ljubljana, Slovenia were studied. Only one isolate from each patient was analyzed. Nineteen isolates were from patients with a urinary tract infection, 3 isolates were from decubiti, 2 isolates were from wound infections, 2 isolates were from surgical wound infections, 2 isolates were from foot ulci and one isolate was from a genital tract infection. All patients were older than 14 years. For cultivation of strains Luria Bertani medium or agar were used.

Detection of PAIs and iron uptake systems

The primers and PCR conditions used to amplify PAI markers; PAI I_{536}, PAI II_{536}, PAI III_{536}, PAI IV_{536}, PAI I_{J96}, PAI II_{J96}, PAI I_{CFT073} and PAI II_{CFT073}, and iron uptake genes; *iutA* – ferric aerobactin receptor gene, *iucD* – lysine: N^6-hydroxylase gene (aerobactin biosynthesis), *iroN* – catecholate siderophore (ferric salmochelin, ferric 2,3-dihydroxybenzoic acid, ferric 2,3-dihydroxybenzoyl-D-ornithine) receptor gene, *iroCD* – salmochelin ATP-binding cassette ABC transporter gene (*iroC*), ferric salmochelin esterase gene (*iroD*), *fyuA* – ferric yersiniabactin receptor gene, *irp2* – yersiniabactin synthetase (yersiniabactin biosynthesis), *iha* – gene for a bifunctional protein: catecholate siderophore (ferric enterobactin, ferric 2,3-dihydroxybenzoylserine) receptor and adhesin, *ireA* – putative TonB-dependent siderophore receptor,

Table 2: Oligonucleotide primers and PCR conditions to detect PAIs and iron uptake systems
Tabela 2: Oligonukleotidni začetniki in pogoji PCR za ugotavljanje PAI in genov za sisteme za privzem železa

Target	Oligonucleotide name and sequence (5' to 3')	Size of product (kbp)	PCR conditions			Reference
PAI I_{536}	I.9	1,8	94°C	5 min	1×	(Sabate & al. 2006)
	TAATGCCGGAGATTCATTGTC		94°C	1 min		
	I.10		56°C	1 min	30×	
	AGGATTTGTCTCAGGGCTTT		72°C	1 min		
			72°C	10 min	1×	
PAI II_{536}	orfIup	1,0	94°C	5 min	1×	(Sabate & al. 2006)
	CATGTCCAAAGCTCGAGC		94°C	1 min		
	orfIdown		62°C	1 min	30×	
	CTACGTCAGGCTGGCTTT		72°C	1 min		
			72°C	10 min	1×	
PAI III_{536}	sfaAI1	0,2	94°C	5 min	1×	(Sabate & al. 2006)
	CGGGCATGCATCAATTATCTTTG		94°C	1 min		
	sfaAI2		63°C	1 min	30×	
	TGTGTAGATGCAGTCACTCCG		72°C	1 min		
			72°C	10 min	1×	
PAI IV_{536}	IRP2FP	0,3	94°C	5 min	1×	(Sabate & al. 2006)
	AAGGATTCGCTGTTACCGGAC		94°C	1 min		
	IRP2RP		61°C	1 min	30×	
	TCGTCGGGCAGCGTTTCTTCT		72°C	1 min		
			72°C	10 min	1×	
PAI I_{J96}	papGIf	0,4	94°C	5 min	1×	(Sabate & al. 2006)
	TCGTGCTCAGGTCCGGAATTT		94°C	0,5 min		
	papGIr		57°C	0,5 min	30×	
	TGGCATCCCACATTATCG		72°C	1 min		
			72°C	10 min	1×	
PAI II_{J96}	Hlyd	2,3	94°C	5 min	1×	(Sabate & al. 2006)
	GGATCCATGAAAACATGGTTAATG		94°C	1 min		
	cnf		61°C	1 min	30×	
	GATATTTTTGTTGCCATTGGTTACC		72°C	2,5 min		
			72°C	10 min	1×	
PAI I_{CFT073}	RPAi	0,93	94°C	5 min	1×	(Sabate & al. 2006)
	GGACATCCTGTTACAGCGCGCA		94°C	1 min		
	RPAf		63°C	1 min	30×	
	TCGCCACCAATCACAGCGAAC		72°C	1 min		
			72°C	10 min	1×	
PAI II_{CFT073}	Cft073.2Ent1	0,4	94°C	5 min	1×	(Sabate & al. 2006)
	ATGGATGTTGTATCGCGC		94°C	0,5 min		
	Cft073.2Ent2		56°C	0,5 min	30×	
	ACGAGCATGTGGATCTGC		72°C	1 min		
			72°C	10 min	1×	
iutA	iutA f	0,3	94°C	4 min	1×	(Johnson & al. 1997)
	GGCTGGACATCATGGGAACTGG		94°C	1 min		
	iutA r		68°C	1 min	35×	
	CGTCGGGAACGGGTAGAATCG		72°C	1 min		
			72°C	10 min	1×	

Target	Oligonucleotide name and sequence (5' to 3')	Size of product (kbp)	PCR conditions			Reference
iucD	Aer 1 TACCGGATTGTCATATGCAGACCGT Aer 2 AATATCTTCCTCCAGTCCGGAGAAG	0,6	94°C 94°C 62°C 72°C 72°C	4,5 min 0,5 min 0,5 min 50 sec 10 min	1× 35× 1×	(Yamamoto & al. 1995)
iroN	iroN f AAGTCAAAGCAGGGGTTGCCCG iroN r GACGCCGACATTAAGACGCAG	0,7	94°C 94°C 68°C 72°C 72°C	2,5 min 0,5 min 0,5 min 2 min 10 min	1× 25× 1×	(Johnson & al. 2000)
iroCD	P52-A GGCTGAGAAATATCAACATCCG P52-B ATCGCACATCCGAAGAACGACT	1,0	94°C 94°C 63°C 72°C 72°C	2,5 min 0,5 min 1 min 1 min 10 min	1× 30× 1×	This study
fyuA	fyuA 1 TGATTAACCCCGCGACGGGAA fyuA 2 CGCAGTAGGCACGATGTTGTA	0,8	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 & Stell 2000, Schubert & al. 1998)
irp2	Irp2 f AAGGATTCGCTGTTACCGGAC Irp2 r TCGTCGGGCAGCGTTTCTTCT	0,3	94°C 94°C 61°C 72°C 72°C	5 min 1 min 1 min 1 min 8 min	1× 35× 1×	(Schubert & al. 1998)
iha	iha f CTGGCGGAGGCTCTGAGATCA iha r TCCTTAAGCTCCCGCGGCTGA	0,8	94°C 94°C 58°C 72°C 72°C	4 min 0,5 min 0,5 min 1 min 8 min	1× 30× 1×	(Johnson & al. 2000)
ireA	ireA f TGGTCTTCAGCTATATGG ireA r ATCTATGATTGTGTTGGT	0,4	94°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×	(Russo T. A. & al. 2001)
hbp	Hbp f GGTGAAGGTACGCTGACGGT Hbp r GCGTGACGCTGGAGTTATCT	0,9	94°C 94°C 65°C 72°C 72°C	4,5 min 0,5 min 1 min 1 min 10 min	1× 35× 1×	This study

and *hbp* – hemoglobin protease autotransporter gene with polymerase chain reaction (PCR) are listed in Tab. 2. DNA to be amplified was released from whole organisms by boiling according to 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).

General molecular biology DNA techniques

Plasmid isolation, DNA digestion and agarose gel electrophoresis were performed by standard methods (Sambrook & Russell 2001).

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 and the level of significance was set at a P value < 0.05.

Results

Prevalence of PAIs, plasmids and iron uptake systems

The PAI with the highest prevalence among the studied ExPEC isolates was PAI IV_{536}, also designated HPI (high-pathogenicity island), which was found in 19 studied strains. PAI II_{CFT073} was found in 6 strains, PAI I_{CFT073} in 4 strains and PAI II_{J96} in only one isolate. The PAIs: PAI I_{536}, PAI II_{536}, PAI III_{536} and PAI I_{J96} were not detected in any studied isolate.

In 19 strains plasmids were detected, among them 9 harboured a plasmid larger than 30 kbp.

The iron uptake system with the highest prevalence was yersiniabactin, the *irp2* gene coding for the yersiniabactin synthetase was found in 20 strains and the *fyuA* gene coding for the ferric yersiniabactin receptor was found in 19 strains. The aerobactin iron uptake system genes *iucD*, coding for lysine: N^6-hydroxylase needed in aerobactin biosynthesis, and *iutA*, encoding the ferric aerobactin receptor, were detected in 12 strains. The *iha* gene was found in 9 strains. The salmochelin uptake system genes *iroN*, coding for the catecholate siderophore receptor, and *iroCD* coding for proteins needed in salmochelin transport, were found in 8 and 7 isolates, respectively. The *ireA* gene was harboured by 7 studied strains and the *hbp* gene by 4 isolates.

Distribution of PAIs and iron uptake systems among strains

As seen from Tab. 3 most of the strains harbouring PAI possessed one PAI (12 strains) and 7 strains harboured 2 or 3 PAIs. In 10 strains none of tested PAIs could be detected. The average PAI number per strain was 1.03.

The majority of tested strains possessed 1 to 3 iron uptake systems (22 strains). In 4 strains none of the tested iron uptake systems could be detected. Three strains possessed four or five different iron uptake systems. The average iron uptake system number per strain was 1.97.

Associations of PAIs and iron uptake systems

Since many PAIs are known to carry iron uptake systems (see Tab. 1), associations of PAIs and iron uptake systems were analyzed. As seen from Tab. 4, only 3 statistically significant associations of PAIs with iron uptake systems could be determined: the yersiniabactin siderophore system was associated with PAI IV_{536}, the aerobactin siderophore system and Iha were associated with PAI I_{CFT073}.

Discussion

Our findings showed that PAIs, plasmids and iron uptake systems are abundant, as the majority of the tested isolates harboured PAIs, plasmids and iron uptake systems.

Since we analysed a relatively small number of isolates it is difficult to compare our results with results obtained by other authors on larger collections of strains, such as the study of SABATE & al. (2006) on the prevalence of PAIs among 100 UPEC strains and 50 commensal strains and the

Table 3: PAIs, iron uptake systems and plasmids in studied strains
Tabela 3: PAI, sistemi za privzem železa in plazmidi v preučevanih sevih

Strain	Patient's diagnosis[a]	Patient's gender[b]	PAI								Iron uptake system									Plasmid (bp)
			I_{536}	II_{536}	III_{536}	IV_{536}	I_{J96}	II_{J96}	I_{CFT073}	II_{CFT073}	*iutA*	*iucD*	*iroN*	*iroCD*	*fyuA*	*irp2*	*iha*	*ireA*	*hbp*	
DL2	UTI	F	–	–	–	+	–	–	–	+	–	–	+	+	+	+	–	+	–	>30.000
DL6	UTI	M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
DL7	UTI	F	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	<30.000
DL8	UTI	F	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
DL14	UTI	F	–	–	–	+	–	–	–	–	+	+	+	+	+	+	–	+	+	>30.000
DL17	UTI	F	–	–	–	+	–	–	–	–	+	–	–	–	+	+	–	–	–	<30.000
DL22	UTI	M	–	–	–	+	–	–	–	–	–	–	–	–	+	+	–	–	+	<30.000
DL37	UTI	F	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	<30.000
DL41	UTI	F	–	–	–	+	–	–	–	–	–	–	–	–	+	+	–	–	–	–
DL43	UTI	F	–	–	–	+	–	–	–	–	–	–	–	–	+	+	–	+	–	–
DL46	UTI	F	–	–	–	+	–	–	–	–	+	+	–	–	+	+	+	–	–	<30.000
DL48	UTI	F	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
DL56	UTI	F	–	–	–	+	–	–	–	–	+	+	–	–	+	+	+	+	–	>30.000
DL76	UTI	F	–	–	–	+	–	–	–	–	–	–	–	–	+	+	+	–	–	>30.000
DL81	UTI	F	–	–	–	+	–	–	–	–	–	–	–	–	+	+	–	–	–	<30.000
DL84	UTI	F	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	>30.000
DL108	UTI	F	–	–	–	+	–	–	–	+	–	–	+	+	+	+	–	+	–	<30.000
DL109	UTI	F	–	–	–	+	–	–	–	–	+	+	–	–	+	+	–	–	+	>30.000
DL110	UTI	F	–	–	–	+	–	+	–	–	+	+	+	+	+	+	–	+	–	>30.000
TA10	WI	F	–	–	–	+	–	–	–	–	–	+	+	–	+	+	–	–	+	–
TA49	WI	F	–	–	–	+	–	–	+	+	+	+	–	–	+	+	+	–	–	–
TA50	D	M	–	–	–	+	–	–	+	+	+	+	–	–	+	+	+	–	–	>30.000
TA71	SWI	F	–	–	–	+	–	–	+	+	+	+	–	–	+	+	+	–	–	–
TA74	GTI	M	–	–	–	+	–	–	–	–	–	–	–	–	+	+	–	–	–	–
TA103	SWI	M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	>30.000
TA160	SU	M	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	<30.000
TA171	D	F	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–	–	<30.000
TA174	UC	M	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–
TA212	D	M	–	–	–	+	–	–	+	+	+	+	–	–	+	+	+	–	–	<30.000

[a]UTI – urinary tract infection, WI – wound infection; D – decubitus; SWI – surgical wound infection; SU – skin ulcus; UC – ulcus cruris
[b]F – female; M – male

Table 4: Associations of PAIs with iron uptake systems
Tabela 4: Povezave PAI in sistemov za privzem železa

	PAI (no. of strains)							
Iron uptake system	**IV_{536} +(19)**	**IV_{536} – (10)**	**II_{J96} +(1)**	**II_{J96} –(28)**	**I_{CFT073} +(4)**	**I_{CFT073} –(25)**	**II_{CFT073} +(6)**	**II_{CFT073} –(23)**
Aer	9	2	1	10	4	7*	4	7
Sal	4	3	1	6	0	7	2	5
Yer	19	0***	1	18	4	15	6	13
Iha	7	2	0	9	4	5*	4	5
IreA	6	1	1	6	0	7	2	5
Hbp	4	0	0	4	0	4	0	4

P values (Fisher's exact test) of statistically significant associations (P<0.05) are indicated by asterisks. Symbols: *, $P < 0.05$; **, $P < 0.005$; ***P < 0.0005. Aer, aerobactin siderophore system; Sal, salmochelin siderophore system; Yer, yersiniabactin siderophore system.

Statično značilna vrednost *P* (Fisherjev eksaktni test) < 0,05 je nakazana z zvezdicami: simboli *, $P < 0,05$; **, $P \leq 0,01$; ***, $P \leq 0,001$. Aer, aerobaktinski sideroforni sistem; Sal, salmohelinski sideroforni sistem; Yer, jersiniabaktinski sideroforni sistem.

study of JOHNSON & al. (2005) on the prevalence of several virulence factors, including *iha*, *fyuA* and *iutA*, among 83 cystitis and 170 pyelonephritis *E. coli*. A further difficulty for comparison of our results with others' is the fact that differences might also be due to geographical differences. Differences in virulence factor profiles between distinct populations have previously been reported among cat populations from distant locations on feline uropathogenic *E. coli* strains from the United Kingdom and feline uropathogenic *E. coli* strains from New Zealand (FREITAG & al 2005). However, it is interesting that among all our studied strains none carried PAI I_{536}, PAI II_{536}, or PAI III_{536} and that among the UTI strains included in this study no strain carrying PAI I_{CFT073} could be detected, while SABATE & al. (2006) reported detection of all PAIs except PAI I_{J96}. The overall lower prevalence in our study could be due to the fact, that our study incorporated only 29 strains.

In our study 19 ExPEC strains harboured at least one plasmid. Since it is commonly known that plasmids are abundant in all bacterial species, the obtained prevalence is of no surprise. It is also not unexpected, that 9 strains in our study harboured large plasmids (>30 kbp), since virulence factors enabling a strain to cause pathogenicity as well as antibiotic resistances can be encoded on large plasmids.

The prevalence of iron uptake system in our study is comparable to data presented in studies on cystitis *E. coli* strains (JOHNSON & al. 2005, KANAMARU & al. 2003). However, uroseptic and pyelonephritic *E. coli* isolates have a higher prevalence of iron uptake systems (JOHNSON & STELL 2000), (JOHNSON & al. 2005), also this is not unexpected since uroseptic and pyelonephritic strains are in general more virulent and possess more virulence factors.

It is known that iron uptake systems can be encoded either chromosomally, sometimes as a part of a genomic island, or on plasmids. The yersiniabactin siderophore system is known to be carried on the HPI (=PAI IV_{536}) (Schmidt & HENSEL 2004) and in our study the association of PAI IV_{536} with this siderophore system was highly statistically significant, all 19 strains encoding the yersiniabactin siderophore system also harboured PAI IV_{536}. The aerobactin siderophore system is known to be part of the PAI I_{CFT073} (SCHMIDT & HENSEL 2004), but it was also found to be carried on a plasmid (CARBONETTI & WILLIAMS 1984). In our study all 4 strains that possessed the PAI I_{CFT073} also harboured the aerobactin siderophore genes, and their association proved to be statistically significant. However, in 7 strains that encoded the aerobactin siderophore system, PAI I_{CFT073} could not be detected, but all 7 strains harboured plasmids large enough to carry the aerobactin siderophore

system therefore, we could assume that the aerobactin siderophore system is more often encoded on plasmids than chromosomally. The same was found for the salmochelin siderophore system. This siderophore system can also be carried by a PAI, the PAI III_{536} (Schmidt & Hensel 2004), but also on a plasmid (Sorsa & al. 2003). In our study in 7 strains we detected the salmochelin siderophore system however, none of these strains harboured the PAI III_{536} and 6 strains carried plasmids large enough to encode the salmochelin siderophore system. The *iha* is known to be part of the PAI I_{CFT073} (Schmidt & Hensel 2004) and in our study 4 out of 9 strains possessing *iha* also possessed the PAI I_{CFT073} (the association was statistically significant). However, the other 5 *iha* encoding strains did not harbour the PAI I_{CFT073}, in 4 of them plasmids large enough to encode *iha* were found. To our knowledge it has not yet been reported that *iha* could be plasmid encoded. The *ireA* and *hbp* were never associated with PAIs, *ireA* was found to be chromosomally encoded (Russo & al. 2001) and *hbp* was found to be plasmid encoded (Otto & al. 1998). In 6 from 7 *ireA*-encoding strains and in 3 from 4 *hbp*-encoding strains plasmids were detected, so we might assume that both *ireA* as well as *hbp* could be either chromosomally encoded or carried by plasmids however, further studies to confirm the location of *iha*, *ireA* and *hbp* genes are needed.

To our knowledge this is the first study of the prevalence of PAIs in a collection of ExPEC strains that included not only UPEC isolates, but also isolates from other extraintestinal infections. Thus, this is the first report of UPEC associated PAIs, PAI I_{CFT073} and PAI II_{CFT073}, that were originally found in UPEC strain CFT073 isolated from blood and urine of a woman with pyelonephritis (Schmidt & Hensel 2004), in strains isolated from skin and soft tissue infections. Further, to our knowledge this is the first study on molecular epidemiology including more than 4 iron uptake systems in ExPEC strains and it is worth to be emphasized that this is the first report on the association of iron uptake systems with PAIs and plasmids. However, since in this study only 29 ExPEC isolates were investigated, further studies examining a large number of ExPEC strains should be performed.

Conclusions

To summarize and conclude:

1. 29 ExPEC strains were screened with PCR for the presence of well characterized PAIs and iron uptake systems as well as with molecular biology techniques for the presence of plasmids;
2. the prevalence, the distribution and the genetic associations of the tested PAIs and iron uptake systems were determined;
3. PAI IV_{536} was found in 19, PAI II_{CFT073} in 6, PAI I_{CFT073} in 4, PAI II_{J96} in 1 of the studied isolates, while PAI I_{536}, PAI II_{536}, PAI III_{536} and PAI I_{J96} were not detected in any studied isolate;
4. in 19 of the studied isolates plasmids were detected;
5. *irp2* was found in 20, *fyuA* in 19, *iucD* and *iutA* in 12, *iha* in 9, *iroN* in 8, *iroCD* in 7, *ireA* in 7 and *hbp* in 4 of the studied isolates;
6. PAI IV_{536} was statistically significantly associated with the yersiniabactin siderophore system and PAI I_{CFT073} was statistically significantly associated with the aerobactin siderophore system and Iha;
7. to our knowledge this is the first report on PAIs and iron uptake systems in an ExPEC collection including isolates from skin and soft tissue infections.

Povzetek

Bakterija *Escherichia coli* (*E. coli*) je najpogostejši fakultativni anaerob med človeškimi črevesnimi mikrobioti. Kljub temu, da je *E. coli* komenzalna bakterija, lahko določeni sevi, ki so pridobili genske zapise za virulentne dejavnike, povzročajo zelo širok spekter okužb, tako črevesnih kot zunajčrevesnih. Genski zapisi za virulentne dejavnike so pogosto vezani – umeščeni v otoke patogenosti (PAI), ki predstavljajo podskupino genomskih otokov. Genski zapisi za virulentne dejavnike pa se pogosto nahajajo tudi v plazmidih, v izvenkromosomskih molekulah DNA. Železo je pomemben element, saj nastopa kot kofaktor v mnogih metabolnih poteh tako gostitelja kot mikroorganizma. Ker je v gostitelju malo prostega železa, imajo mikrobi različne

sisteme za privzem železa, med najbolj znanimi so t. i. sideroforji. Naša raziskava je vključevala 29 sevov zunajčrevesnih patogenih *E. coli* (ExPEC), osamljenih na Inštitutu za mikrobiologijo in imunologijo Medicinske fakultete v Ljubljani iz diagnostičnih vzorcev 19 bolnikov z urinarnimi infekcijami, 9 bolnikov z infekcijami kože in podkožja in 1 bolnika z infekcijo spolovil. S pomočjo PCR smo preučevane seve preiskali za prisotnost različnih otokov patogenosti, ki so jih prvotno našli in opisali v uropatogenih sevih *E. coli* (PAI I_{536} do PAI IV_{536}, PAI I_{J96}, PAI II_{J96}, PAI I_{CFT073} in PAI II_{CFT073}) in sisteme privzema železa (gena *iutA* in *iucD* aerobaktinskega sideroformega sistema, geni *iroN*, in *iroCD* salmohelinskega sideroformega sistema, gena *fyuA* in *irp2* jersiniabaktinskega sideroformega sistema, gen *iha* receptorja kateholatnega sideroforja Iha, gen *ireA* od TonB odvisnega sideroformega receptorja IreA in gen *hbp* hemoglobinske proteaze Hbp). Nadalje, smo z molekulskobiološkimi tehnikami preverjali prisotnost plazmidov v preučevanih sevih. Ugotavljali smo prevalenco in povezave preučevanih PAI in sistemov za privzem železa. PAI IV_{536} smo našli v 19, PAI II_{CFT073} v 6, PAI I_{CFT073} v 4 , PAI II_{J96} v 1 od preučevanih izolatov. PAI I_{536}, PAI II_{536}, PAI III_{536} in PAI I_{J96} nismo odkrili v nobenem izmed preučevanih izolatov. V 19 sevih smo odkrili plazmide, v 9 sevih smo našli plazmide, ki so bili večji od 30 kb. Gen *irp2* smo našli v 20, *fyuA* v 19, *iucD* in *iutA* v 12, *iha* v 9, *iroN* v 8 , *iroCD* v 7, *ireA* v 7 in *hbp* v 4 izmed preučevanih sevov. PAI IV_{536} je bil statistično značilno povezan s sideroformim sistemom jersiniabaktin in PAI I_{CFT073} je bil statistično značilno povezan s sideroformim sistemom aerobaktin in Iha. Kolikor nam je poznano je to prva raziskava o PAI in sistemih za privzem železa na zbirki sevov ExPEC, ki vključuje tudi izolate iz infekcij kože in podkožja.

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