Journal of Infection and Public Health 13 (2020) 1537–1543

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

Original Article

Virulence, phylogenetic background and antimicrobial resistance in *Escherichia coli* associated with extraintestinal infections

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ARTICLE INFO

Article history:
Received 22 January 2020
Received in revised form 5 May 2020
Accepted 25 June 2020

Keywords:
E. coli extraintestinal infection
Virulence
Phylogenetic groups
Antimicrobial resistance

ABSTRACT

Background: Escherichia coli (*E. coli*) is a major cause of urinary tract infections and bloodstream infections and an important agent in the resistance to antibiotics. The present study sought to determine associations between virulence, phylogenetic background and antimicrobial resistance of *E. coli* strains isolated from patients with extraintestinal infections.
Methods: A total of three hundred ten *E. coli* strains were isolated from blood, skin and soft tissue and urine. PCR methods were used to detect four main phylogenetic groups (A, B1, B2 and D) and 11 virulence genes (3 toxins, 3 adhesins, 1 siderophore, 4 capsule synthesis proteins and protectins). Standard broth microdilution test was used to determine sensitivity to 12 antimicrobial drugs.
Results: The most common and the most virulent phylogenetic group B2 was found in 193 (62.3%) isolates. The lowest virulence was observed among the group A. Analysis of virulence factors revealed the *kpsMTII* gene in 212 (68.4%), *aer* in 194 (62.6%) and *tra* in 184 (59.4%) of isolates, respectively. Multi-drug resistant (MDR) phenotype was noticed in 165 (53.2%) isolates. Lower representation of the MDR phenotype was detected in *E. coli* containing all groups of virulence genes and in the avirulent *E. coli*.
Conclusions: Our study documented that *E. coli* associated with 3 different extraintestinal infections contain various virulence factors. Genes *afa*, *pap*, *aer*, *neuC* show significant differences among the 3 groups of the strains tested and might be the prerequisite virulence factors in bloodstream infections. Isolates containing all groups of virulence genes predominantly originate in the blood and belong to the B2 phylogenetic group. Overall, we identified significantly higher incidence of all the groups of virulence genes examined among the B2 group. Prevalence of the MDR phenotype and high levels of resistance to ampicillin, ciprofloxacin and trimetoprim/sulfamethoxazole reflect the trend observed worldwide in recent years.

Introduction

Escherichia coli is normal inhabitant of the intestine of humans and various animals. However, some strains have been described to cause infections of the gastrointestinal system (intestinal pathogenic *E. coli*) while others cause infections outside the gastrointestinal system (extraintestinal pathogenic *E. coli*, ExPEC) [1,2]. ExPEC are implicated in large number of extraintestinal infections such as urinary tract infections (UTI), bloodstream infections, pneumonia, skin and soft tissue infections, and neonatal meningitis [3]. *E. coli* remains the leading cause of UTI, with recent investigations reporting the emergence of *E. coli* as the predominant cause of nosocomial and neonatal sepsis infections [4].

Phylogenetic studies have shown that *E. coli* falls into four main phylogenetic groups namely A, B1, B2, and D. Human ExPEC strains belong generally to the more virulent group B2, and in a lesser extent, to group D. Commensal strains, considered less virulent, belong to A or B1 groups [5]. ExPEC strains possess a broad range of virulence factors (VFs) responsible for pathogenesis that are frequently encoded by pathogenic islands and other mobile DNA

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https://doi.org/10.1016/j.jiph.2020.06.032

elements [6]. VFs of ExPEC include toxins, adhesins, iron acquisition systems (siderophores), capsule synthesis proteins, protectins and invasins [7].

Adhesins (e.g. P-fimbriae, S-fimbriae) allow ExPEC to bind to specific receptors, which enable the pathogen to adapt to different host tissues during the infection initiation [8]. Siderophores (aerobactin) contribute to bacterial survival and growth outside the gut [9]. Toxins (e.g. hemolysin, cytotoxic necrotizing factor) damage human cells and tissues [10]. The capsule synthesis proteins and protectins are important in prevention of phagocytosis and complement-mediated killing of ExPEC. Genes *traT, kps MT II, neuC, rfc* and other encode proteins involved in these processes of pathogenesis. They are essential for survival of *E. coli* localized in urinary tract and bloodstream [11,12].

Until the late 1990s, ExPEC were highly susceptible to the first line antibiotics (ATB). However several surveillance studies during the 2000s across the world have shown increased resistance to the first line antibiotics including cephalosporins, fluoroquinolones, and trimetoprim-sulfamethoxazole [13]. The appearance and increase of antimicrobial resistance among the ExPEC constitutes a major obstacle to the treatment and is implicated in increasing numbers of hospitalizations and deaths and increasing healthcare costs associated with ExPEC infections [14].

The acquisition of resistance or virulent traits may represent a survival advantage to the organism. Many antimicrobial genes are inserted in conjugative plasmids that may also carry virulence factors determinants, and might be selected by antibiotic selective pressure. Furthermore, stable virulent clones or strains may be perpetuated if they acquire resistance determinants [1,15].

The aim of this study was to compare the distribution of virulence factors, phylogenetic groups and antimicrobial resistance among three groups of extraintestinal *E. coli* strains isolated from blood, skin and soft tissue and urine and to determine the association between phylogenetic groups and resistance, resistance and virulence, phylogenetic groups and virulence of tested *E. coli* strains.

Material and methods

Bacterial isolates and DNA extraction

A total of 310 non-repetitive *E. coli* isolates were obtained from inpatients who presented with extraintestinal infections in University Hospital Košice, Slovakia, during January 2016 to June 2018. Isolates were recovered from three different clinical specimens, including blood, urine, and skin and soft tissue (SST). The group Blood (n = 130) contained strains isolated from hemocultures of septicemic patients. The group Urine (n = 115) included strains isolated from urine of patients with acute UTI (n = 94) and chronic UTI (n = 21). In the group SST (n = 65) strains isolated from swabs of patients with skin and soft tissue infections (SSTI) were included. Demographic and clinical characteristics of *E. coli* strains are listed in the Table 1. Automated blood culture system (Bactec 9050) was used in hemocultivation. The identities of all strains were confirmed in our laboratory by MALDI-TOF analysis performed on Microflex Maldi Biotyper (Bruker Daltonik) using a standard protocol. Template DNA was extracted by Bacterial DNA Extraction kit (*Ecoli s.r.o.*, Slovakia).

Phylogenetic classification

Phylogenetic classification of *E. coli* strains was determined by triplex PCR assay described by Clermont et al. [5] using primers targeted at three markers, *chuA*, *yjaA*, and TSPE4.C2 (Table 2). Regarding the presence/absence of these genes *E. coli* strains can be assinged to four main phylogroups: A, B1, B2, or D.

Detection of virulence genes

PCR methods were used to detect the presence of the following virulence genes: toxins (*hlyA*, *cnf1*, *cdt*), adhesins (*afa*, *sfa*, *pap*), siderophores (*aer*), capsule synthesis proteins (CSP) and protectins (*neuC, kpsMT II, rfc, traT*) as described above (Table 2). All testing was done in parallel with relevant positive and negative controls. PCR products were separated in 1.5% agarose gel, visualised using UV imaging system Gel Doc^{TM} EZ System (BIO-RAD) and analyzed using a Image Lab Software (BIO-RAD). A 100 bp ladder (Invitrogen) was used as a molecular size standard.

Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of ampicillin (AMP), piperacillin/tazobactam (PIB), gentamicin (GEN), amikacin (AMI), cefepime (FEP), cefotaxime (CTX), ceftazidime (CAZ), cefuroxime (CXM), meropenem (MEM), trimetoprim/sulfamethoxazole (COT), ciprofloxacin (CIP) and colistin (COL) were determined by MIDITECH colorimetric microdilution test. This system is a modification of the standard broth microdilution method that uses a 3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide dye for bacterial viability detection [19]. Strains were classified as susceptible or resistant to ATB based on the breakpoints for interpretation of MICs issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

E. coli strains were divided into three groups according to their ATB susceptibility: S (susceptible to all of the tested antimicrobial agents), non-MDR (resistant to a member/members of one or two antimicrobial classes) and MDR (multi-drug resistant). MDR was

Table 1
Demographic and clinical characteristics of 310 *E. coli* strains.

	Blood (n = 130) No (%)		SST (n = 65) No (%)		Urine (n = 115) No (%)
Demographics					
Age of < 65 years	43 (33.1)		20 (30.8)		21 (18.3)
Age of > 65 years	87 (66.9)		45 (69.2)		94 (81.7)
Female gender	76 (58.5)		34 (52.3)		89 (77.4)
Male gender	54 (41.5)		31 (47.7)		26 (22.6)
Origin of septicemia		**Origin of SSTI**		**Acute UTI**	
Urinary	35 (26.9)	Surgical-site infection	47 (72.3)	Pyelonephritis	77 (67)
Intrabdominal	34 (26.2)	Abscess	8 (12.3)	Urethritis	13 (11.3)
Gastrointestinal	19 (14.6)	Decubitus ulcer	7 (10.8)	Cystitis	2 (1.7)
Medical procedure	16 (12.3)	Diabetic foot ulcer	3 (4.6)	Prostatitis	2 (1.7)
Biliary tract/liver	10 (7.7)			**Chronic UTI**	
Pulmonary	7 (5.4)			Nephritis	16 (13.9)
Not defined	9 (6.9)			Cystitis	4 (3.5)
				Prostatitis	1 (0.9)

Table 2
Target genes for detection of phylogenetic groups and virulence factors.

Target gene	Encoding product	Size of product (bp)	Reference
chuA	Heme transport in EHEC O157:H7	279	[5]
yjaA	Protein of unkmown function	211	[5]
TSPE4.C2	Anonymous DNA fragment	152	[5]
hlyA	α-hemolysin	1177	[16]
cnf1	Cytotoxic necrotizing factor	498	[16]
cdt	Cytolethal distending toxin	466	[17]
afa	Afimbrial adhesin	750	[16]
sfa	S-fimbriae	410	[16]
pap	P-fimbriae	328	[16]
aer	Aerobactin	602	[16]
neuC	Sialic acid synthesis	676	[18]
kpsMTII	Capsule synthesis	272	[11]
rfc	O4 lipopolysaccharide biosynthesis	788	[11]
traT	SERUM resistance	290	[11]

Table 3
Phylogenetic groups, ATB susceptibility groups and virulence genes (VG) groups in *E. coli* strains.

	Total (n = 310) No (%)	Blood (n = 130) No (%)	SST (n = 65) No (%)	Urine (n = 115) No (%)	Statistics *P*
Phylogenetic groups					
A	33 (10.6)	13 (10)	6 (9.2)	14 (12.2)	–
B1	26 (8.4)	10 (7.7)	5 (7.7)	11 (9.6)	–
B2	193 (62.3)	77 (59.2)	44 (67.7)	72 (62.6)	–
D	58 (18.7)	30 (23.1)	10 (15.4)	18 (15.7)	–
ATB susceptibility groups					
S	76 (24.5)	36 (27.7)	19 (29.2)	21 (18.3)	–
Non-MDR	69 (22.3)	34 (26.15)	13 (20)	22 (19.1)	–
MDR	165 (53.2)	60 (46.15)	33 (50.8)	72 (62.6)	–
VG groups					
All VG groups	38 (12.3)	25 (19.2)	3 (4.6)	10 (8.7)	a)
No VG	26 (8.4)	7 (5.4)	7 (10.8)	12 (10.4)	–

a) Statistical significance: Blood vs. SST (0.0048), Blood vs. Urine (0.0135).

defined as the non-susceptible profile to ≥1 agent in ≥3 antimicrobial categories [20].

Statistical analysis

Statistical assessments were perfomed using IBM SPSS Statistics (version 19). Comparisons of proportions for a particular characteristic between different populations were studied using the Pearson χ^2 test and the two-tailed Fisher's exact test. P values of less than 0.05 were considered statistically significant.

Results

Prevalence of phylogenetic groups

Phylogenetic analysis revealed that 33 (10.6%) isolates belonged to group A, 26 (8.4%) were group B1, 193 (62.3%) were group B2, and 58 (18.7%) belonged to group D (Table 3). Distribution of phylogenetic groups among 3 examined groups of *E. coli* strains was simillar. Statistically significant differences were not detected.

Virulence genes

The presence of studied virulence genes and statistically significant differences are reported in Table 4. The prevalence of three toxin genes ranged from 1.6% (*cdt*) to 19.7% (*hly*). The *afa*, *sfa* and *pap* genes encoding adhesins were detected in 18.4%, 21.3% and 24.8% of isolates, respectively. The *aer* gene was identified in 62.6% of strains. With regard to capsule synthesis proteins and protectins, the *kpsMT II* gene was the most common virulence gene and was detected in 68.4% of isolates. In contrast, the *rfc* gene was present just in 1.3% of isolates. Using a statistical analysis we found significant differences in the presence of *afa*, *pap*, *aer* and *neuC* genes among the studied groups of *E. coli* strains.

Antimicrobial resistance

Seventy-six isolates (24.5%) were susceptible to all tested ATB (Table 3). In contrast, MDR phenotype was noticed in 165 strains (53.2%). The highiest resistance rates were detected among the urine isolates (62.6%), while the swabs isolates were the most susceptible (29.2%).

The resistance to AMP (65.2%) was observed the most frequently followed by CIP (52.9%) and COT (50.7%). All examined *E. coli* strains were sensitive to MEM. The lowest resistance rates were observed in COL (2%) and AMI (12.9%). Significant differences in antimicrobial resistance among the 3 groups of *E. coli* strains were observed in AMI, AMP, PIB, CXM and COL. Detailed results are listed in Table 4.

Association between phylogenetic groups and resistance

The highiest occurrence of MDR strains was observed among the isolates belonging to the phylogenetic group A (57.6%), followed by group B2 (47.3%) (Table 5). *E. coli* isolates sensitive to all tested antimicrobial drugs belonged mainly to groups A (30.3%) and D (29.3%).

Association between resistance and virulence

Among the 310 isolates tested, 38 (12.3%) *E. coli* carried at least one virulence factor from each of four VG groups (toxins, adhesins, siderophores, and CSP and protectins) (Table 3). Majority of these

Table 4
Virulence genes and antimicrobial resistance of *E. coli* strains.

	Total (n = 310) No (%)	Blood (n = 130) No (%)	SST (n = 65) No (%)	Urine (n = 115) No (%)	Blood vs. SST *P*	Blood vs. Urine *P*	SST vs. Urine *P*
Virulence genes							
hlyA	61 (19.7)	26 (20)	11 (16.9)	24 (20.9)	–	–	–
cnf1	56 (18.1)	27 (20.8)	11 (16.9)	18 (15.7)	–	–	–
cdt	5 (1.6)	1 (0.8)	0	4 (3.5)	–	–	–
afa	57 (18.4)	34 (26.2)	14 (21.5)	11 (9.6)	–	0,0009	0,0416
sfa	66 (21.3)	32 (24.6)	11 (16.9)	23 (20)	–	–	–
pap	77 (24.8)	46 (35.4)	11 (16.9)	20 (17.4)	0,0077	0,0016	–
aer	194 (62.6)	98 (75.4)	41 (63.1)	55 (47.8)	–	<0.0001	0,018
neuC	31 (10)	16 (12.3)	3 (4.6)	12 (10.4)	0,0493	–	–
kps MT II	212 (68.4)	95 (73.1)	46 (70.8)	71 (61.7)	–	–	–
rfc	4 (1.3)	2 (1.5)	1 (1.5)	1 (0.9)	–	–	–
tra	184 (59.4)	73 (56.2)	37 (56.9)	74 (64.4)	–	–	–
ATB							
GEN	50 (16.1)	21 (16.2)	8 (12.3)	21 (18.3)	–	–	–
AMI	40 (12.9)	23 (17.7)	11 (16.9)	6 (5.2)	–	0,0015	0,0089
AMP	202 (65.2)	69 (53.1)	44 (67.7)	89 (77.4)	–	<0.0001	–
PIB	44 (14.2)	32 (24.6)	7 (10.8)	5 (4.4)	0,0107	<0.0001	–
FEP	94 (30.3)	33 (25.4)	21 (32.3)	40 (34.8)	–	–	–
CTX	123 (39.7)	46 (35.4)	27 (41.5)	50 (43.5)	–	–	–
CAZ	95 (30.7)	34 (26.2)	20 (30.8)	41 (35.7)	–	–	–
CXM	131 (42.3)	45 (34.6)	32 (49.2)	54 (47)	0,0182	0,0152	–
MEM	0	0	0	0	–	–	–
COT	157 (50.7)	70 (53.9)	28 (43.1)	59 (51.3)	–	–	–
CIP	164 (52.9)	65 (50)	32 (49.2)	67 (58.3)	–	–	–
COL	6 (2)	0	1 (1.5)	5 (4.4)	–	0,0217	–

Table 5
Distribution of ATB susceptibility groups and VG groups among *E. coli* strains according to the phylogenetic classification.

Phylogenetic group					
	A (n = 33) No (%)	B1 (n = 26) No (%)	B2 (n = 193) No (%)	D (n = 58) No (%)	Statistics *P*
ATB susceptibility groups					
S	10 (30.3)	7 (26.9)	42 (17.7)	17 (29.3)	–
Non-MDR	4 (12.1)	11 (43.3)	39 (16.5)	14 (24.1)	a)
MDR	19 (57.6)	8 (30.8)	112 (47.3)	27 (46.6)	b)
VG groups					
Toxins	2 (6.1)	2 (7.7)	66 (34.2)	5 (8.6)	c)
Adhesins	1 (3)	7 (26.9)	129 (66.8)	17 (29.3)	d)
CSP and protectins	10 (30.3)	16 (61.5)	185 (95.9)	52 (89.7)	e)
Siderophores	19 (57.6)	7 (26.9)	141 (73.1)	36 (62.1)	f)
All VG groups	1 (3)	1 (3.8)	34 (17.6)	2 (3.5)	g)
No VG	12 (36.4)	6 (23.1)	3 (1.6)	5 (8.6)	h)

Only statistically significant differences are shown: a) B1–A (0.0146), B1–B2 (0.0222) b) B1–B2 (0.0113) c) B2–A (0.0007), B2–B1 (0.0058), B2–D (<0.0001) d) B2–A (<0.0001), B2–B1 (0.0002), B2–D (<0.0001), A–B1 (0.0168), A–D (0.0021) e) A–B1 (0.0201), A–B2 (<0.0001), A–D (<0.0001), B1–B2 (<0.0001), B1–D (0.0052) f) B1–A (0.0337), B1–B2 (<0.0001), B1–D (0.0043) g) B2–A (0.0352), B2–B1 (0.0454), B2–D (0.005) h) B2–A (<0.0001), B2–B1 (0.0001), B2–D (0.0178), A–D (0.0018).

strains (44.7%) were susceptible to all tested ATB (Graph 1). On the other hand, 26 (8.4%) *E. coli* strains with no presence of VG were detected (Table 3), 57.7% of them were fully antimicrobial susceptible. MDR isolates were found in both groups (with all VG groups and no VG) in 26.3% and 23.1%, respcetively (Graph 1).

Association between phylogenetic groups and virulence

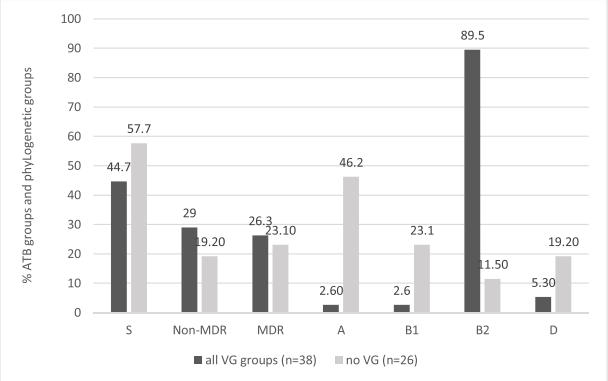
According to the phylogenetic classification, *E. coli* strains belonging to the phylogenetic group B2 possessed more virulence genes, as compared to those from groups A, B1, and D (Table 5). Moreover, 33 (17.1%) of these multivirulent B2 strains carried at least one virulence gene from all VG groups. The lowest virulence was observed among the strains from group A, 36.4% of these isolates were found to harbour any of investigated VG. Statistical analysis revealed multiple significant differences in the presence of VG groups among the groups A, B1, B2 and D (Table 5).

When comparing *E. coli* strains with all VG groups, phylogenetic group B2 was predominat (89.1%). Among the 26 *E. coli* strains without presence of VG (no VG), group A was the most prevalent (46.2%), followed by groups B1 and D (both 19.1%) (Graph 1).

Discussion

ExPEC is one of the leading causes of urinary tract and bloodstream infections worldwide. The pathogenicity of these bacteria may depend on a variety of virulence determinants [21]. The importance of ExPEC in terms of public health has been increased after recognition of multiple antimicrobial resistances in different phylogenetic groups. In the present study, we screened 310 *E. coli* strains from blood, urine and skin and soft tissue for the determination of phylogenetic group, antimicrobial resistance, and important virulence factors responsible for extraintestinal pathogenesis.

Phylogenetic analysis revealed that *E. coli* isolates causing 3 clinical syndromes were predominately phylogenetic groups B2 and



Graph 1. ATB susceptibility groups and phylogenetic groups (%) of *E. coli* isolates with all VG groups vs. no VG.

D (62.3% and 18.7%), which are considered virulent *E. coli* [22]. Higher prevalence of phylogenetic group B2 has been described in extraintestinal *E. coli* strains isolated from blood [23], urine [24] and in set of *E. coli* strains isolated from SSTI, respiratory infections, intra-abdominal infections and genital smears [25]. The presence of virulence genes belonging to CSP and protectins, siderophores, adhesins and toxins has been mostly associated with B2 group, whereas less virulent strains belonged to the A and B1 groups. Results of antimicrobial susceptibility testing revealed lower percentage of fully susceptible and non-MDR strains in B2 group, while significantly higher proportion of MDR strains was found in A group.

E. coli strains isolated from blood were significantly enriched with virulence factors from each functional category studied. As iron is limiting in the bloodstream, iron acquisition systems are important VFs and very common in bloodstream isolates [21,26]. Our results showed high incidence of aerobactin encoding sequences (75.4%). Of the genes that confer serum resistance, *traT*, which encodes outer membrane lipoprotein contributing to serum resistance was detected in 56.2% of isolates and *kpsMTII*, which encodes capsule in 73.1%. Finding a high prevalence of serum resistance factors among the strains is feasible because the strains were isolated from blood cultures where only serum-resistant pathogens could survive [27]. Of the 3 toxin genes, *cnf1* and *hlyA* were prevalent genes in our strains from blood. Hemolysin is a pore-forming exotoxin that may contribute to the virulence of bacteria during bloodstream infection and sepsis [28]. Cytotoxic necrotizing factor induces a severe controlled inflammatory response [29]. Moreno et al. [26] have suggested that *E. coli* causing bacteremia of urinary tract origin need toxins to invade and remain in the bloodstream. The majority of our *cnf1* and *hlyA* positive strains originate from urinary tract as well. Adhesins are characteristic extraintestinal virulence factors that play a role in the initiation of infection. The incidence of 3 adhesin genes ranged from 24.6% to 35%. The importance of adhesins is supported by the fact that 80 of 130 blood isolates owned at least one of these genes (data not shown). The highest incidence of 8 virulence genes among the studied groups of *E. coli* indicates that this group of *E. coli* is the most virulent. Our statement is supported by determination of VG groups in *E. coli* from blood. We revealed 25 (19.2%) strains containing all groups of VG and 7 (5.4%) strains with no VG group.

Strains of uropathogenic *E. coli* (UPEC) participate as the dominant agents of both acute and chronic UTI. The initial step in the pathogenesis of UPEC is adhesion mediated by several adhesins produced according to the stage of infection [30]. Adhesin genes were found in 44 of 115 strains isolated from urine, most of them were B2 group (data not shown). The most common adhesin gene *sfa* (20%) was observed predominantly in strains isolated from patients with acute pyelonephritis and chronic nephritis. The expression of other genes encoding important virulence factors (e.g. *aer*, *pap*, *afa*, *sfa*, *hlyA*, *kpsMT*, *traT*) contributes to disease severity and is known to be involved in pathogenicity of this organism [31]. The most common gene of urine isolates, *traT* (64.4%), outlines their virulence potential. Among toxins, α-hemolysin is cytolytic toxin that is produced by up 50% of UPEC isolates. It has been associated with clinical severity in UTI patients [32]. In our study, *hlyA* was detected in 20.9% of urine strains isolated predominantly from patients with pyelonephritis. Cytolethal distending toxin can be produced by intestinal and extrainstestinal pathogenic *E. coli*. The *cdt* genes are spread among *E. coli* isolates with wide spectrum of virulence genes. A coincidence was found between *cdt* and *cnf1* and other virulence genes in ExPEC [33]. We detected 4 *cdt* positive strains from urine. All these strains possessed genes *traT* and *kpsMTII*, 3 strains possessed *sfa* and 2 strains *cnf1*, *hlyA*, *pap*. Among the isolates from blood, one *cdt* positive strain containing *kpsMTII*, *cnf1*, *hlyA*, *sfa* was found. Results of our 5 strains point to coincidence between *cdt* and *kpsMTII* in ExPEC.

E. coli strains isolated from skin and soft tissue predominantly originate from surgical-site infections (72.3%), rarely from abscesses, decubitus ulcers and diabetic foot ulcers. Compared to *E. coli* strains from blood and urine, strains isolated from SST exhibited lower prevalence of sequences encoding α-hemolysin (16.9%), cytolethal distending toxin (0%), S-fimbriae (16.9%), P-fimbriae (16.9%), and sialic acid synthesis protein (4.6%). When focusing on VG groups in SST *E. coli,* we found only 3 strains containing all VG groups but 7 strains with no VG. These results may suggest lower virulent potential of SST *E. coli*. On the other hand, these strains presented the highest incidence of phylogenetic group B2 (67.7%) among 3 tested groups and high incidence of *kpsMTII* (70.8%) and *traT* (56.9%). Research realized by Petkovsek et al. [34] revealed higher proportion of *cnf1*, *hlyA*, *pap*, *sfa* and similar proportion of *kpsMTII* and phylogenetic groups. The group of ExPEC isolated from SST is not very well characterized but according to these authors virulence potential of *E. coli* isolated from SSTI differs from both intestinal pathogens and commensal strains and would be simillar to that of other ExPEC strains.

In this study, we investigated ExPEC strains and their susceptibility patterns to different antimicrobial drugs that are commonly administered to treat the infections. The studied isolates presented 53.2% incidence of MDR phenotype, making them the causative agent of a critical health problem. Many previous works from different regions point to the same problem. The rate of MDR in UPEC strains was 52.7% in Turkey [9], 62.2% in Thailand [35], 62.6% in our research, the rate of MDR in blood strains was 40% in Ireland [27], 50.7% in England [36] and 46,15% in our research. The results of another study from France and Spain show a better situation in the incidence of MDR, where only 37.2% ExPEC strains isolated from urine, blood, bile and other sources demonstrated MDR phenotype [37]. The high levels of resistance of *E. coli* strains to AMP (65.2%), CIP (52.9%) and COT (50.7%) were observed in our study. Comparing to our results, Flament-Simon et al. [37] reported resistance to AMP, CIP and COT in 56.6%, 21.4% and 29.1% of tested strains, respectively. These data may reflect a higher rate of application of these ATB to all types of infections, leading to increased resistance in our region. When focusing on the strains isolated from urine, AMP, CIP and COT resistance rates correspond to those of other studies realized in Thailand [35] and Turkey [9]. In the group of blood isolates, COT resistance rate was simillar to the study of Bozcal et al. in Turkey [38], while AMP resistance rate was lower and CIP resistance rate higher than those of other studies from Turkey and Ireland [38,27]. Difference between levels of resistance was observed in our isolates from urine, blood and SST, where the

resistance to GEN, AMP, FEP, CTX, CAZ, CIP, COL was the highest in isolates from urine, the resistance to AMI and COT in isolates from blood and the resistance to CXM in isolates from SST. Almost all these resistant strains have been reported in patients over 65 years of age (data not shown). The high degree of resistance in urine isolates may be result of empirical antibiotic treatment started in patients with suspected UTI or of the long-term exposure to various antimicrobials for strains causing asymptomatic bacteriuria. The ExPEC resistance to a diverse set of antimicrobials is increasing. This trend is clearly visible when comparing antimicrobial resistance in one region over a period of several years. Prevalence of MDR phenotype in our urine isolates was 62.6% but in Koreň et al. [39] only 31.7%. SST and blood isolates exhibited higher incidence of fully susceptible and non-MDR phenotype than urine isolates. The reason for this is probably targeted therapy of SSTI and sepsis.

Among the 310 isolates tested, 38 *E. coli* with at least one virulence gene from each of four VG groups and 26 *E. coli* with no presence of VG have been detected. *E. coli* strains with all VG groups have been isolated predominantly from blood (n = 25). Analysis of the origin among these strains indicated that primary disease in combination with high virulence may play a significant role in the development of sepsis. The most common primary disease was UTI (n = 11), followed by intraabdominal infections (n = 5) and medical procedure (n = 5). *E. coli* strains with no VG were isolated mostly from urine (n = 12). The spectrum of acute and chronic UTI among these strains was different, no association between primary disease and zero virulence was observed. Interestingly, 6 of these strains were fully susceptible to antimicrobials and belonged to phylogenetic groups A (n = 4), B1 (n = 1) and D (n = 1). All these isolates were obtained from patients over 65 years of age. These findings indicate that the normal human digestive flora represents a potential reservoir, which can spread to urinary tract. The combination of older age, various diseases and weakening of the immune system provides for commensal strains the opportunity to assert themselves as pathogens.

When focusing on the phylogenetic background of these 38 strains, *E. coli* containing all groups of virulence genes has been found significantly predominant in group B2 and the isolates with no VG belonged predominantly to group A. Results of antimicrobial sensitivity testing revealed S phenotype to be most common in both groups. This may suggest that close relationship between the genetic diversity of *E. coli* and environmental factors provides the ability to adapt to different conditions and niches in the host's body. These changes can affect the shift of *E. coli* from commensal to pathogenic strain responsible for extraintestinal infection [40].

In conclusion, the results of this study indicate that ExPEC strains associated with sepsis, skin and soft tissue infections and urinary tract infections belong predominantly to the phylogenetic group B2 and contain various virulence factors. Some factors (*afa*, *pap*, *aer*, *neuC*) exhibit significant differences among the 3 groups of tested strains and appear to be critical for bloodstream infections. *E. coli* strains containing all groups of virulence genes predominantly originate from the blood. Generally, presence of the virulence genes examined, belonging to CSP and protectins, siderophores, adhesins and toxins, was significantly higher in the B2 strains, which highlights pathogenic potential of these *E. coli.* Prevalence of MDR phenotype and high levels of resistance to AMP, CIP and COT especially among isolates from urine reflect the trend observed worldwide in recent years. Lower representation of the MDR phenotype observed in *E. coli* strains containing all groups of virulence genes and avirulent *E. coli* strains may be the result of high plasticity of the bacterial genome. The ability of such strains to cause the disease still does not depend on the bacteria's genetic potential but rather on more factors contributing to the establishment of infectious process, such as the immune status of the patient, environmental conditions, etc. . .

Funding

This study was supported by project VERZDRAV (ITMS: 26220220197).

Competing interests

None declared.

Ethical approval

Human subjects and patient specific data were not included in this study. ExPEC isolates were provided from Clinical Microbiology Laboratory within routine diagnosis, not for purposes of this study. For this reason, ethical explicitness was not necessary.

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