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Virulence Potential of *Escherichia coli* Isolates from Skin and Soft Tissue Infections[▽]

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***Escherichia coli* strains frequently are isolated from skin and soft tissue infections (SSTI); however, their virulence potential has not yet been extensively studied. In the present study, we characterized 102 *E. coli* SSTI strains isolated mostly from surgical and traumatic wounds, foot ulcers, and decubitus. The strains were obtained from the Institute of Microbiology and Immunology, University of Ljubljana, Slovenia. Phylogenetic backgrounds, virulence factors (VFs), and antibiotic resistance profiles were determined. Correlations between VFs and phylogenetic groups were established and analyzed with regard to patient factors. Further, the associations of the three most prevalent antibiotic resistance patterns with virulence potential were analyzed. Our results showed that the majority of the studied strains (65%) belonged to the B2 phylogenetic group. The most prevalent VF was *ompT* (80%), while toxin genes *cnf1* and *hlyA* were found with prevalences of 32 and 30%, respectively. None of the investigated bacterial characteristics were significantly associated with patient gender, age, type of infection, or immunodeficiency. The most prevalent antibiotic resistance pattern was resistance to ampicillin (46%), followed by resistance to tetracycline (25%) and fluoroquinolones (21%). Strains resistant to ciprofloxacin exhibited a significantly reduced prevalence of *cnf1* ($P < 0.05$) and *usp* ($P < 0.01$). Our study revealed that *E. coli* isolates from SSTIs exhibit a remarkable virulence potential that is comparable to that of *E. coli* isolates from urinary tract infections and bacteremia.**

Skin and soft tissue infections (SSTIs) are one of the most common infections in patients of all age groups. Infections mostly are self limited or can be treated with antibiotics. However, moderate or severe cases may require hospitalization and parenteral therapy (30). The most common causative agents are *Staphylococcus aureus* and aerobic streptococci (9, 10, 41, 43). However, several reports associating the enterobacterium *Escherichia coli* with SSTI have been published: *E. coli* was found to be the causative agent of neonatal omphalitis (7), cellulitis localized to lower or upper limbs (4, 6, 49), necrotizing fasciitis (1, 25, 28), surgical site infections (44), infections after burn injuries (37), and others. A study monitoring SSTIs during a 7-year period and encompassing three continents (Europe, Latin America, and North America) showed *E. coli* to be an important causative agent, since it was the third-most prevalent isolated species, preceded solely by *S. aureus* and *Pseudomonas aeruginosa.* The beta-hemolytic *Streptococcus* sp. group was only the 7th-ranked pathogen in North America and Europe and was 10th in Latin America in terms of prevalence (30). *E. coli* isolates from SSTI therefore merit detailed studies, especially taking into account the dramatic decline in antibiotic susceptibility of pathogenic *E. coli* strains in recent years. Despite the need for the characterization of *E. coli* strains from SSTI, to our knowledge only a single *E. coli* isolate from a deep surgical wound infection has been characterized (21). The aim of our study was to characterize a larger collection of *E. coli* isolates from SSTI. Our work was focused on their virulence potential: phylogenetic distribution, virulence factor (VF) profile, and the prevalence of antibiotic resistance patterns. Correlations between patient and strain characteristics were studied, as well as correlations between VF profiles and antibiotic resistance. As the strains were isolated from extraintestinal sites of infections, we screened for VFs that are typical of extraintestinal pathogenic *E. coli* (ExPEC).

MATERIALS AND METHODS

Strains. The studied *E. coli* strains were isolated between 28 August 2006 and 30 November 2006 at the Institute of Microbiology and Immunology, Medical Faculty, Ljubljana, Slovenia. During this time period, 3,186 wound samples and swabs of eyes, ears, and genital infections from patients residing in different parts of Slovenia were collected. *E. coli* strains were found in 216 (7%) of the collected samples. One hundred two (47%) of the *E. coli*-positive samples were from SSTIs, 38 (37%) were from surgical wounds, 20 (20%) were from foot ulcers, 14 (14%) were from decubitus, 9 (9%) were from traumatic wounds, 5 (5%) were from localized skin infections in the perigenital/perianal region, 5 (5%) were from fistulae (2 fistulae were perianal, 1 was inguinal, and 2 were from skin at the site of radiotherapy), 4 (4%) were from omphalitis, 4 (4%) were from diabetic wounds, 1 (1%) was from a burn infection, 1 (1%) was from a radiation wound, and 1 (1%) was from a pustule. Thirteen (13%) *E. coli* strains were from monomicrobial SSTI, and 89 (87%) *E. coli* strains were from polymicrobial SSTI. Fifty-eight (57%) of the isolates were obtained from male patients, and 44 (43%) were from female patients. The mean age of the patients was 51.1 years; 20 (20%) of the patients were children younger than 19 years, 14 (14%) were between 19 and 45, 26 (25%) were between 46 and 65, and 42 (41%) patients were older than 65 years. In 63 (62%) cases the infection was acute, and in the remaining 39 (38%) the infection was chronic. The infection was regarded as chronic if (i) the wound remained unhealed for at least 3 months or if the diagnosis was either (ii) foot ulcer, (iii) decubitus, or (iv) diabetic gangrene. Patients were regarded as immunocompromised if they (i) suffered from a chronic wound and were older than 65 years, (ii) were neonates (aged up to 4 weeks), (iii) suffered from diabetes and a wound(s), (iv) were undergoing treatment for cancer at the Institute of Oncology, Ljubljana, or (v) were undergoing

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TABLE 1. VF prevalence among phylogenetic groups[a]

VF or no. of VFs	Prevalence of trait (no. [%] of isolates) by phylogenetic group				
	All isolates (n = 102 [100%])	A (n = 12 [12%])	B1 (n = 10 [10%])	B2 (n = 66 [65%])	D (n = 14 [14%])
Toxins					
cnf1	33 (32)	4 (33)	2 (20)	22 (33)	5 (36)
hlyA	31 (30)	3 (25)	2 (20)	21 (32)	5 (36)
Fimbriae and/or adhesins					
papA	43 (41)	3 (25)	1 (10)	36 (55)*	3 (21)
papGII	10 (10)	0	0	9 (14)	1 (7)
papGIII	15 (15)	2 (17)	0	11 (17)	2 (14)
sfaDE	37 (36)	2 (17)	4 (40)	25 (38)	6 (43)
afa/draBC	1 (1)	0	0	1 (2)	0
Iron uptake					
iucD	48 (47)	7 (58)	3 (30)	34 (52)	4 (29)
Capsule					
kpsMT	66 (65)	6 (50)	4 (40)	48 (73)	8 (57)
Other					
ompT	82 (80)	7 (58)	6 (60)	58 (88)	11 (79)
usp	45 (44)	4 (33)	3 (30)	35 (53)	3 (21)
No. of VFs					
0	7 (7)	1 (8)	3 (30)	2 (3)	1 (7)
1	10 (10)	4 (33)	1 (10)	2 (3)	3 (21)
2	11 (11)	0	1 (10)	8 (12)	2 (14)
3	13 (13)	1 (8)	2 (20)	10 (15)	0
4	23 (23)	3 (25)	0	16 (24)	4 (29)
5	18 (18)	2 (17)	2 (20)	12 (18)	2 (14)
6	10 (10)	0	1 (10)	7 (11)	2 (14)
7	6 (6)	1 (8)	0	5 (8)	0
8	4 (4)	0	0	4 (6)	0

[a] A correlation between a VF and the phylogenetic group B2 was ascertained by comparing the rates of prevalence of VFs between B2 and all non-B2 strains. Fisher's exact test and Bonferroni's correction were used to analyze the data. The P value after Bonferroni's correction is indicated by an asterisk, where $P < 0.01$.

treatment at the Institute for Rehabilitation, Ljubljana, and suffered from decubitus for more than 6 months. Fifty-three (52%) of the patients were immunocompromised. A single strain from each patient was analyzed.

Phylogenetic analysis. The isolates were assigned to one of the four main phylogenetic groups (A, B1, B2, or D) by multiplex PCR, as described by Clermont et al. (5).

VF profiling. All isolates were tested for the following VFs: *cnf1* (cytotoxic necrotizing factor 1), *hlyA* (hemolysin), *papA*, *papGII*, and *papGIII* (P fimbriae), *sfaDE* (S fimbriae), *afa/dra* (Afa/Dr adhesins), *iucD* (aerobactin), *kpsMT* (group II capsule), *ompT* (outer membrane protease), and *usp* (uropathogenic specific protein). Prior to genotyping, boiled lysates were prepared (27). The PCR mix (25 μl) contained template DNA (5 μl), primers at a concentration of 0.8 μM, deoxynucleoside triphosphate (0.2 mM), $MgCl_2$ (2.5 mM), and *Taq* DNA polymerase (5 U/μl) in 1× *Taq* DNA buffer. The primers and PCR programs used were described previously (14, 19, 22, 23, 26, 27, 32, 48). Dot blot hybridization was performed to confirm the PCR results. Positive and negative controls were included. To calculate the VF score, alleles *papA*, *papGII*, and *papGIII* were considered as a single *pap* VF. Thus, if a strain was positive for at least one of the studied *pap* alleles, it was regarded as *pap* positive.

Antimicrobial susceptibility testing. Antimicrobial sensitivity testing to the following antimicrobial agents was performed by the estimation of MICs using Etest (AB Biodisk) and the National Committee for Clinical Laboratory Standards (NCCLS) interpretive criteria (33): ampicillin, piperacillin, amoxicillin plus clavulanic acid, piperacillin plus tazobactam, cefazolin, cefuroxime axetil, cefoxitin, cefixime, cefotaxime, ceftazidime, cefepime, imipenem, aztreonam, gentamicin, amikacin, netilmicin, tetracycline, norfloxacin, ciprofloxacin, trimethoprim plus sulfamethoxazole, and ertapenem.

Statistical analysis. Fisher's exact test (two-tailed) (http://www.langsrud.com/fisher.htm) and Bonferroni's correction were used to analyze the data. The threshold for statistical significance after Bonferroni's correction was set at $P < 0.05$.

RESULTS

Phylogenetic groups. Our analysis showed that the majority of the studied isolates, namely 66 strains (65%), belonged to group B2. Fourteen isolates (14%) were assigned to group D, 12 (12%) to group A, and 10 (10%) to group B1.

VFs. The most prevalent VF among our isolates was *ompT*, which was found in 82 (80%) of the tested strains, followed by *kpsMT*, which was detected in 66 (65%) strains (Table 1). Other VFs were found in less than 50% of the isolates. The toxin genes *cnf1* and *hlyA* were found in approximately one-third of the strains: *cnf1* in 33 (32%) and *hlyA* in 31 (30%) strains. Twenty-seven (26% of all strains, 82% of strains carrying *cnf1*, and 87% of strains carrying *hlyA*) strains encoded both toxins. The most prevalent adhesin sequences among our isolates were P fimbriae; namely, 44 (43%) of the tested strains carried at least one of the amplified *papA* or *papG* alleles. Only one isolate was found to encode *afa/draBC* of Afa/Dr adhesins (1%). The analysis of the two P fimbrial allelic variants for the binding adhesin (*papGII* and *papGIII*) showed that both alleles were encoded with similar prevalence levels, namely 10 strains (10%) and 15 strains (15%), respectively (Table 1). The analysis of the major rod subunit *papA* showed that the F10 allele was by far the most prevalent, as it was found in 26 isolates, representing 60% of the strains that carried a *papA* allele (data

TABLE 2. Distribution of phylogenetic groups, VFs, and number of VFs in relation to patient gender, age, infection type, and immune system status[a]

Phylogenetic group, VF, or no. of VFs	Prevalence (no. [%] of the tested strains)									
	Sex		Age				Infection type		Immune system status	
	Male (n = 58 [57%])	Female (n = 44 [43%])	<19 yr (n = 19 [19%])	19–45 yr (n = 15 [15%])	46–65 yr (n = 26 [25%])	>65 yr (n = 42 [41%])	Acute (n = 63 [62%])	Chronic (n = 39 [38%])	Normal (n = 49 [48%])	Weakened (n = 53 [52%])
Phylogenetic group										
A	5 (9)	7 (16)	2 (11)	3 (20)	4 (15)	3 (7)	9 (14)	3 (8)	7 (14)	5 (9)
B1	5 (9)	5 (11)	2 (11)	2 (13)	3 (12)	3 (7)	5 (8)	5 (13)	3 (6)	7 (13)
B2	41 (71)	25 (57)	13 (68)	7 (47)	16 (62)	30 (71)	41 (65)	25 (64)	31 (63)	35 (66)
D	7 (12)	7 (16)	2 (11)	3 (20)	3 (12)	6 (14)	8 (13)	6 (15)	8 (16)	6 (11)
VF										
cnf1	16 (28)	17 (39)	8 (42)	6 (40)	7 (27)	12 (29)	25 (40)	8 (21)	13 (27)	20 (38)
hly	17 (29)	14 (32)	6 (32)	4 (27)	8 (31)	13 (31)	23 (37)	8 (21)	13 (27)	18 (34)
papA	21 (36)	22 (50)	5 (26)	4 (27)	12 (46)	22 (52)	23 (37)	20 (51)	21 (43)	22 (42)
papGII	5 (9)	5 (11)	1 (5)	0	5 (19)	4 (10)	7 (11)	3 (8)	2 (4)	8 (15)
papGIII	6 (10)	9 (20)	1 (5)	4 (27)	4 (15)	6 (14)	11 (17)	4 (10)	10 (20)	5 (9)
sfaDE	22 (38)	15 (34)	9 (47)	7 (47)	10 (38)	11 (26)	25 (40)	12 (31)	17 (35)	20 (38)
afa/dra	1 (2)	0	1 (5)	0	0	0	1 (1)	0	1 (2)	0
iucD	29 (50)	19 (43)	8 (42)	8 (53)	11 (42)	21 (50)	29 (46)	19 (49)	21 (43)	27 (51)
kpsMT	37 (64)	29 (66)	14 (74)	11 (73)	12 (46)	29 (69)	41 (65)	25 (64)	25 (51)	41 (77)
ompT	45 (78)	37 (84)	15 (79)	14 (93)	17 (65)	36 (86)	49 (78)	33 (85)	40 (82)	42 (79)
usp	23 (40)	22 (50)	10 (53)	9 (60)	9 (35)	17 (40)	29 (46)	16 (41)	20 (41)	25 (47)
No. of VFs										
0	4 (7)	3 (7)	1 (5)	0	4 (15)	2 (5)	4 (6)	3 (8)	4 (8)	3 (6)
1	7 (12)	1 (2)	2 (11)	1 (7)	4 (15)	3 (7)	5 (8)	5 (13)	6 (12)	4 (8)
2	7 (12)	4 (9)	2 (11)	2 (13)	2 (8)	5 (12)	8 (13)	3 (8)	8 (16)	3 (6)
3	7 (12)	5 (11)	1 (5)	3 (20)	2 (8)	7 (17)	7 (11)	6 (15)	5 (10)	8 (15)
4	13 (22)	10 (23)	5 (26)	3 (20)	8 (31)	7 (17)	15 (24)	8 (21)	9 (18)	14 (26)
5	10 (17)	8 (18)	3 (16)	2 (13)	1 (4)	12 (29)	9 (14)	9 (23)	8 (16)	10 (19)
6	5 (9)	5 (11)	3 (16)	1 (7)	2 (8)	4 (10)	9 (14)	1 (3)	6 (12)	4 (8)
7	2 (3)	4 (9)	1 (5)	3 (20)	1 (4)	1 (2)	3 (5)	3 (8)	2 (4)	4 (8)
8	3 (5)	1 (2)	1 (5)	0	2 (8)	1 (2)	3 (5)	1 (3)	1 (2)	3 (6)

[a] A correlation between bacterial and patient characteristics was ascertained using Fisher's exact test and Bonferroni's correction. No statistically significant correlations were found.

not shown). Eight strains (19% of the *papA* coding strains) carried allele F12 or F15. The *papA* allele F9 was found in five strains (12%), alleles F12, F13, and F16 in four strains (9%), alleles F11 and F14 in three strains (7%), allele F8 in two strains (4%), and the F7-2 allele in one strain (2%). The *papA* allelic variants F7-1, F15, and F48 were not found in any of the studied strains. In one isolate the *papG* gene was detected, whereas *papA* analysis was negative for all tested alleles.

The number of VFs detected in a single strain varied from zero to eight. The investigated strains most commonly possessed three to five VFs. The average number of VFs (average VF score) was 3.8 (data not shown).

Distribution of VFs among phylogenetic groups. The distribution of the VFs of the studied strains with regard to phylogenetic group B2 is presented in Table 1. In general, strains belonging to the B2 phylogenetic group exhibited the highest prevalence of VFs, although only the association of *papA* with the B2 group was statistically significant ($P < 0.01$) (Table 1).

Subsequently, the correlation between the VF score and the phylogenetic group was examined. The majority of strains encoding six to eight VFs belonged to the B2 phylogenetic group. Strains encoding three to five VFs were more equally distributed among all phylogenetic groups. The presence of zero to two of the tested VFs was uncommon among the group B2 strains.

Distribution of phylogenetic groups and VFs in relation to patient characteristics. The distribution of virulence potential-associated characteristics in relation to patient gender, age (younger than 19, 19 to 45, 46 to 65, or older than 65), infection type (acute or chronic), and immune system status (normal or weakened) was analyzed. For comparisons of phylogenetic groups, individual VFs, and VF scores in relation to patient characteristics, not a single statistically significant association was found (Table 2).

The distribution of bacterial characteristics in relation to the clinical syndrome was analyzed for isolates from surgical wound infections, and no significant correlations were found (data not shown). Other syndrome subgroups, as well as the number of monomicrobial *E. coli* strains, were too small to perform a statistical analysis.

Antimicrobial resistance. The resistance of the studied isolates to some of the most clinically relevant antibiotics was tested. The most prevalent, found in 47 (46%) isolates, was resistance to ampicillin, followed by tetracycline resistance and resistance to fluoroquinolones (norfloxacin and ciprofloxacin), which was found in 25 (25%) and 21 (21%) isolates, respectively. Nineteen (19%) of the strains were resistant to piperacillin, 17 (17%) to trimethoprim plus sulfamethoxazole, and 14 (14%) to amoxicillin plus clavulanic acid. Nine (9%) strains were resistant to cefazolin, a cephalosporin of the narrow spec-

TABLE 3. Distribution of phylogenetic groups, VFs, and numbers of VFs in relation to resistance phenotypes[a]

Phylogenetic group, VF, or no. of VFs	Prevalence (no. [%] of strains)					
	Tetracycline		Ciprofloxacin		Ampicillin	
	Susceptible (n = 75 [74%])	Resistant (n = 25 [25%])	Susceptible (n = 80 [78%])	Resistant (n = 21 [21%])	Susceptible (n = 55 [54%])	Resistant (n = 47 [46%])
Phylogenetic group						
A	7 (9)	5 (20)	9 (11)	3 (14)	6 (11)	6 (13)
B1	7 (9)	3 (12)	7 (9)	3 (14)	4 (7)	6 (13)
B2	52 (69)	12 (48)	52 (65)	13 (62)	40 (73)	26 (55)
D	9 (12)	5 (20)	12 (15)	2 (10)	5 (9)	9 (19)
VF						
cnf1	26 (35)	7 (28)	32 (40)*	1 (5)	18 (33)	15 (32)
hlyA	28 (37)	3 (12)	29 (36)	2 (10)	18 (33)	13 (28)
papA	34 (45)	8 (32)	32 (40)	11 (52)	25 (45)	18 (38)
papGII	10 (13)	0	9 (11)	1 (5)	6 (11)	4 (9)
papGIII	11 (15)	3 (12)	13 (16)	2 (10)	9 (16)	6 (13)
sfaDE	33 (44)	4 (16)	33 (41)	4 (19)	24 (44)	13 (28)
afa/draBC	1 (1)	0	1 (1)	0	1 (2)	0
iucD	33 (44)	14 (56)	35 (44)	13 (62)	25 (45)	23 (49)
kpsMT	52 (69)	13 (52)	53 (66)	12 (57)	36 (65)	30 (64)
ompT	64 (85)	17 (68)	69 (86)	12 (57)	48 (87)	34 (72)
usp	39 (52)	5 (20)	43 (54)**	2 (10)	26 (47)	19 (40)
No. of VFs						
0	3 (4)	3 (12)	3 (4)	4 (19)	2 (4)	5 (11)
1	5 (7)	5 (20)	7 (9)	3 (14)	5 (9)	5 (11)
2	7 (9)	4 (16)	9 (11)	1 (5)	6 (11)	5 (11)
3	11 (15)	2 (8)	9 (11)	4 (19)	7 (13)	6 (13)
4	17 (23)	6 (24)	18 (23)	5 (24)	13 (24)	10 (21)
5	14 (19)	3 (12)	14 (18)	4 (19)	8 (15)	10 (21)
6	10 (13)	0	10 (13)	0	8 (15)	2 (4)
7	4 (5)	2 (8)	6 (8)	0	4 (7)	2 (4)
8	4 (5)	0	4 (5)	0	2 (4)	2 (4)
Avg virulence score	4.1	2.9	4.1	2.7	4.0	3.5

[a] Two strains were not tested for tetracycline resistance, and one strain was not tested for ciprofloxacin resistance. These strains therefore were omitted from susceptibility/resistance analysis for the two antibiotics. *P* values, determined using Bonferroni's correction, are indicated with asterisks: *, $P < 0.05$; **, $P < 0.01$.

trum, while the prevalence of resistance to cephalosporins of the expanded spectrum (cefuroxime axetil and cefoxitin), broad spectrum (cefixime, cefotaxime, and ceftazidime), and "fourth generation" (extended spectrum) (cefepime) was 5% or lower. The least prevalent resistance patterns were resistance to piperacillin plus tazobactam, aztreonam, and gentamicin, as only one strain (1%) was found to be resistant. A single strain was found to produce extended-spectrum beta-lactamase. All studied strains were susceptible to imipenem, amikacin, netilmicin, and ertapenem.

Association of antimicrobial resistance with virulence potential. A correlation between virulence potential and antibiotic susceptibility/resistance was studied for tetracycline, ciprofloxacin, and ampicillin, the three most prevalent antibiotic resistance patterns among the studied isolates. We found that susceptible strains more commonly (albeit not significantly) belonged to the B2 phylogenetic group, and that the prevalence of VFs among these strains was higher (Table 3), as the VF score was higher. The difference in VF score between susceptible and resistant strains was 1.2 for tetracycline-susceptible/resistant strains and 1.4 for ciprofloxacin-susceptible/resistant strains, while it was 0.5 in the case of ampicillin susceptibility/resistance. Strains resistant to ciprofloxacin harbored *cnf1* ($P < 0.05$) and *usp* ($P < 0.01$) significantly less frequently. No other significant differences were found (Table 3).

Twelve (12%) of the studied isolates were resistant to both tetracycline and ciprofloxacin, and 67 strains (66%) were susceptible to both antibiotics. Virulence profile analysis showed that the strains resistant to both tetracycline and ciprofloxacin possessed the fewest VFs, as they possessed 2.3 VFs on average (data not shown). *ompT* was found to be negatively associated with resistance to both tetracycline and ciprofloxacin ($P < 0.05$). In general, VFs were concentrated in strains susceptible to both antibiotics, as the average VF score of the susceptible strains reached a value of 4.2 (data not shown). Statistically significant correlations between susceptible strains and the possession of *hlyA* ($P < 0.05$) and *usp* ($P < 0.01$) sequences were found. Strains resistant to a single antibiotic reached a VF score of 3.5 (Cip^r) and 3.6 (Tc^r) (data not shown).

DISCUSSION

Even though *E. coli* is the most frequently isolated enterobacterium from SSTI, to our knowledge this is the first study of the virulence profile and antibiotic susceptibility of a larger collection of SSTI *E. coli* strains. Since the studied strains were

TABLE 4. Prevalence of phylogenetic groups and VFs in our and previous studies[i]

Phylogenetic group or VF	Prevalence (no. [%] of isolates)									
	SSTI from present study (n = 102)	Sample type from previous study								
		Meningitis[a] (n = 70)	Recurrent cystitis[b] (n = 74)	UTI[c] (n = 93 women)	Vagina[d] (n = 88)	Bacteremia[e] (n = 63)	UTI[f] (n = 377)	Acute cystitis[g] (n = 100)	Rectal isolates[e] (n = 71)	Human feces[h] (n = 266)
Phylogenetic group										
A	12 (12)	1 (1)*		7 (8)	7 (8)	7 (11)		20 (20)	11 (15)	52 (20)
B1	10 (10)	7 (10)		3 (3)	0**	2 (3)		6 (6)	13 (18)	33 (11)
B2	66 (65)	57 (81)		64 (69)	67 (76)	42 (67)		55 (55)	38 (54)	120 (45)**
D	14 (14)	5 (7)		19 (20)	14 (16)	12 (19)		19 (19)	9 (13)	61 (23)
VF										
cnf1	33 (32)	6 (9)**	25 (34)	26 (28)	17 (19)	23 (37)		34 (34)	9 (13)*	43 (16)**
hly	31 (30)	6 (9)**	27 (36)	31 (33)	19 (22)	28 (44)		44 (44)	10 (14)	51 (19)
papA	43 (41)		26 (35)					46 (46)		
papGII and *papGIII*	24 (24)	15 (21)	27 (36)			43 (68)***		48 (48)**	19 (27)	
sfa	37 (36)	41 (59)	23 (31)	26 (41)	18 (20)	8 (13)*		6 (11)***	5 (7)***	
afa/dra	1 (1)	18 (26)***	3 (4)	17 (18)***	5 (6)	3 (5)		14 (14)**	4 (6)	14 (5)
aer (iucD/iutA)	48 (47)	43 (61)	21 (28)	46 (49)	31 (35)	34 (54)			14 (20)**	86 (32)
kpsMT	66 (65)	60 (86)*	46 (62)	76 (82)		50 (79)	342 (91)***	69 (69)	34 (48)	142 (53)
ompT	82 (80)	67 (96)*		81 (87)		51 (81)	354 (94)**	53 (53)***	11 (15)***	155 (58)***
usp	45 (44)						320 (85)***			

[a] Data are from reference 20.
[b] Data are from reference 18.
[c] Data are from reference 50.
[d] Data are from reference 34.
[e] Data are from reference 39.
[f] Data are from reference 24.
[g] Data are from reference 16.
[h] Data are from reference 11.
[i] Statistical significance of differences in prevalence was calculated between our strain collection and every single ExPEC collection presented in the table. P values, determined using Bonferroni's correction, are indicated with asterisks: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

isolated from extraintestinal sites of infection, we assumed that their virulence potential would be similar to that of other ExPEC strains. ExPEC strains differ significantly from both intestinal pathogens and commensal strains, as they possess typical virulence determinants (adhesins such as P fimbriae, iron acquisition systems such as aerobactin, host defense avoidance mechanisms such as capsule, and toxins such as hemolysin) and belong mainly to phylogenetic group B2 or, to a lesser extent, group D (36, 38).

An assessment of our results and those of other studies of ExPEC and human fecal *E. coli* isolates is presented in Table 4. Compared to fecal isolates, the strains investigated in our study more frequently belonged to the B2 phylogenetic group and exhibited a higher prevalence of the tested VFs. In addition, a VF profile similar to that of other ExPEC subgroups is evident. Nonetheless, some discrepancies in the prevalence of individual VFs are obvious, namely, the prevalence of *usp* among SSTI isolates was somewhat lower, 44%, while Bauer et al. (2) reported a prevalence of 69% for urinary tract infection (UTI) strains, 74% for periurethral isolates, and 58% for vaginal isolates. Previously, in an experimental mouse model, the USP protein was shown to contribute to UTI (47). Therefore, the observed lower levels of prevalence among non-UTI isolates are not surprising. On the other hand, discrepancies also might be due to geographic differences in the distribution of VFs. Differences in VF profiles between distinct populations previously have been reported among cat populations from distant locations. Among feline uropathogenic *E. coli* strains from the United Kingdom, a 41% prevalence of *papGII* and *papGIII* was determined, while among feline uropathogenic *E. coli* strains from New Zealand the prevalence of *papGII* and *papGIII* was 100% (8).

It is noteworthy that the rates of the prevalence of *cnf1* and *hlyA* (32 and 30%, respectively) were similar to those found among UTI isolates (Table 4), indicating that CNF1 also could play a significant role in SSTIs. While it is well established that CNF1 is a urotoxin, a recent study showed that in vitro CNF1 also blocks intestinal epithelial wound repair (3). *cnf1* is known to be associated with *hlyA* in pathogenicity island II J96 (40); therefore, the high prevalence of *hlyA* (31%) probably is due to the presence of pathogenicity island sequences, as more than 80% of the strains harbored both toxin genes *cnf1* and *hlyA*. Whether hemolysin, a well established urotoxin, is important for instigating SSTIs has, to our knowledge, not yet been investigated. The same can be stated for all other tested VFs.

Further, new as-yet unidentified VFs could play a significant role in SSTIs. For example, our study indicates that new *papA* alleles are harbored by some isolates, since from a strain that encoded *papG* adhesins no *papA* alleles could be amplified in the multiplex PCR designed by Johnson et al. (23).

It is well established that host characteristics can play a decisive role in the development of disease. To ascertain whether there is an association between patient characteristics and the virulence potential of the studied strains, we analyzed gender, age, infection type, and immune system status in relation to virulence potential. No significant differences were observed. However, further studies of larger strain collections are needed, especially as in our study we could not distinguish patients that are immunocompromised due to treatment with steroids and neutropenics.

The resistance of pathogens to antimicrobial agents is a global health care problem and a subject of intense research. A number of studies have reported a significantly reduced virulence potential among uropathogenic *Escherichia coli* isolates that are resistant to certain antibiotics, such as quinolones, chloramphenicol, tetracycline, and others (12, 15, 17, 29, 31, 42, 45, 46), but not among ampicillin- and trimethoprim-resistant isolates (16, 46). In our study, the most prevalent resistances were to ampicillin, tetracycline, norfloxacin, ciprofloxacin, piperacillin, trimethoprim plus sulfamethoxazole, and amoxicillin plus clavulanic acid. These antibiotics were, or still are, of high clinical significance, and therefore higher resistance prevalences are not surprising. The virulence potential in relation to antibiotic susceptibility/resistance was analyzed for tetracycline, ciprofloxacin, and ampicillin, as resistances to these three antibiotics were the most prevalent. The results of our analysis are in agreement with previous studies performed on UTI isolates (13, 31, 35, 42), demonstrating a lower virulence potential among strains that are resistant to tetracycline and ciprofloxacin, while ampicillin-resistant strains exhibited no loss in virulence potential. Several hypotheses have been postulated to explain such differences in VF profiles (15, 29, 35, 46); however, currently it is believed that the differences observed between the VF profiles of antibiotic-susceptible and -resistant strains are due to differences in population sources. It has been suggested that ecological factors determine associations, and that antibiotic-resistant strains derive from an animal or environmental source rather than from human fecal *E. coli* (15, 16). An exception is ampicillin-resistant isolates, which may derive from susceptible strains via the acquisition of transferable resistance elements, with no major changes in the VF profile (16).

In conclusion, the studied *E. coli* strains from SSTIs exhibited a remarkable virulence potential, indicating their medical significance. Even though additional, in vivo studies should be performed to confirm the significance of the detected VFs, we believe that *E. coli* strains should be considered important causative agents of SSTIs. The further analysis of VF profiles with regard to specific clinical syndromes and defined severity is recommended.

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