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Distribution of pathogenicity island markers in commensal and uropathogenic *Escherichia coli* isolates

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Abstract Uropathogenic *Escherichia coli* (UPEC) isolates contain large genomic segments, termed pathogenicity islands (PAIs), that contribute to their virulence. A total of 150 UPEC and 50 commensal *E. coli* isolates from outpatients were investigated for antimicrobial susceptibility and the presence of eight PAI markers. One hundred ninety (95 %) isolates were resistant to one or more antimicrobial agents. The most frequent resistance found against amoxicillin (68 %), amoxicillin/clavulanic acid (55 %), aztreonam (50 %), trimethoprim/sulfamethoxazole (46 %) and tetracycline (43.5 %). Antimicrobial resistance among UPEC isolates was higher than that of commensals. PAI markers were detected in substantial percentage of commensal (88 %) and UPEC isolates (98.6 %) ($P>0.05$). The most prevalent PAI marker among UPEC and commensal isolates was PAI IV_{536} (98.7 % UPEC vs. 84 % commensal). We found a high number of PAI markers such as PAI I_{CFT073}, PAI II_{CFT073}, PAI I_{536}, PAI II_{536}, PAI III_{536} and PAI II_{J96} significantly associated with UPEC. PAI III_{536} (21.3 %) and PAI II_{J96} (8 %) were detected only in the uropathogenic isolates. Several different combinations of PAIs were found among UPEC isolates. Comparison of PAIs among UPEC and commensal isolates showed that many UPEC isolates (79.3 %) carried two or more PAI markers, while 6 % of commensals had two PAI markers ($P<0.05$). The most frequent combinations of PAI markers in UPEC isolates were PAI IV_{536}+PAI II_{CFT073} (18 %) and PAI IV_{536}+PAI I_{CFT073}+PAI II_{CFT073} (18 %). These results indicate that PAI markers are widespread among commensal and UPEC isolates and these commensal isolates may be reservoirs for transmission of these markers.

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Introduction

Escherichia coli is the prototypic commensal species of the facultatively anaerobic microbiota in the human large intestine, but certain strains also cause extraintestinal infections (extraintestinal pathogenic *Escherichia coli*—ExPEC) including urinary tract infections (UTIs), meningitis, pneumonia, skin and soft-tissue infections, and sepsis (Koga et al. 2014; Östblom et al. 2011). Uropathogenic *Escherichia coli* (UPEC) is one of the primary etiological agents of UTIs, accounting for 75–90 % of community-acquired UTIs and approximately 50 % of nosocomial UTIs (Oliveira et al. 2011; Copur-Cicek et al. 2014).

The emergence of antimicrobial resistant *E. coli* has become a serious public health threat worldwide (Rice 2009). The intensive use of antimicrobial agents in human and veterinary medicine is associated with an emerging resistance against therapeutic drugs, followed by the selection of virulence and resistance gene cassettes carrying *E. coli* strains in humans, animals and the environment (Sepp et al. 2009). Horizontal transfer of these gene cassettes seems to be the main cause of the rapid spread of antibiotic-resistance genes across a wide diversity of bacteria. Beyond the horizontal gene transfer, the loss and acquisition of functional modules are important in the process of rapid bacterial development of resistance (Wozniak and Waldor 2010).

Commensal and ExPEC isolates typically differ with respect to phylogenetic groups and virulence attributes

(Sabaté et al. 2006). ExPEC pathogenicity is due to the presence of virulence genes located on chromosome or plasmids which are infrequent among commensal *E. coli* isolates. These virulence genes on the chromosome are typically found in specific regions called pathogenicity islands (PAIs) (Ananias and Yano 2008). Increasing evidence shows that differences in virulence between pathogenic and nonpathogenic bacterial strains can be attributed in part to virulence genes located in PAIs (Wenting et al. 2013). Pathogenicity islands were described for the first time in uropathogenic *E. coli* strain 536 in the late 1980s by Hacker et al. (1990). The virulence determinants encoded on different PAIs of UPEC strains 536, J96 and CFT073 are shown in Table 1. PAIs are distinct genetic elements of pathogens encoding various virulence factors such as protein secretion systems, host invasion factors, iron uptake systems and toxins (Yoon et al. 2005). PAIs are a subset of genomic islands and can be identified by features such as the large size (>10 kb), frequent association with tRNA encoding genes or other *att* sites for temperate bacteriophages, and a G+C content different from host bacterial core genome. These elements are frequently flanked by repeated sequences and carry many fragments of other mobile and accessory genetic elements such as bacteriophages, plasmids and insertion sequence (IS) elements. Some PAIs are unstable regions and can spontaneously disappear from the chromosome. Therefore, PAIs are considered to have evolved from mobile genetic elements by horizontal gene transfer. It can also be assumed that these DNA regions underwent and will continue to undergo further evolutionary changes, resulting in an ongoing evolution of bacterial pathogens (Dobrindt et al. 2002). Identification of PAIs is essential in understanding the development of disease and the evolution of bacterial pathogenesis (Yoon et al. 2005). The objectives of the present case–control study were to compare the presence of various PAI markers among UPEC and commensal *E. coli* isolates from the stools of healthy subjects and to determine the antimicrobial resistance profiles of these isolates.

Materials and methods

Bacterial isolation In this case–control study, between March 2013 and February 2014, a total of 200 *E. coli* strains were isolated from urine and stool specimens of three major university hospitals in Zanjan, Iran. One hundred fifty strains were isolated from urine samples of adult outpatients with symptomatic urinary tract infections (UTIs) and 50 strains from stool specimens of healthy subjects with no history of diarrhoea and antibiotic therapy for at least 1 month (control group). Criteria for symptomatic UTI (all cases of UTIs were cystitis episodes) included dysuria, urgency and frequency of micturition. Patients with fever, nausea and vomiting symptoms, catheterization and those yielding mixed infection were excluded from the study. Specimens were cultured on MacConkey agar (Merck, Germany), and one isolate from each patient was analysed. Verified isolates of *E. coli* were preserved at −70 °C in trypticase soy broth (Merck) containing 20 % (*v*/*v*) glycerol for further analysis.

Antimicrobial susceptibility testing Susceptibility of isolates to the following antibiotics was examined using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute 2013): Amoxicilin (25 μg), Aztreonam (30 μg), Amikacin (30 μg), Cefotaxime (30 μg), Cefoxitine (30 μg), Ceftazidime (30 μg), Ciprofloxacin (5 μg), Amoxicillin/clavulanic acid (30 μg), Trimethoprim/sulfamethoxazole (25 μg), Cefepime (10 μg), Gentamicin (10 μg), Imipenem (10 μg) and Tetracycline (30 μg) (MAST, Merseyside, UK). Isolates shown to be resistant to at least three different classes of antimicrobial agents were determined to be multidrug resistant (MDR). *E. coli* ATCC 25922 was used as control for antibiotic resistance.

Detection of PAI markers in *E. coli* isolates The presence of the eight PAIs, PAI I_{536}, PAI II_{536}, PAI III_{536}, PAI IV_{536},

Table 1 The pathogenicity islands and the functions encoded

PAIs	Virulence determinants	Ref.
PAI I_{536}	α-Haemolysin, CS12 fimbriae and F17-like fimbrial adhesin	Östblom et al. 2011
PAI II_{536}	α-Haemolysin, P-related fimbriae, hemagglutinin-like adhesion, Hek adhesion, P fimbriae, iron-regulated proteins	Östblom et al. 2011
PAI III_{536}	S fimbriae, salmochelin, HmuR-like heme receptor, Sat toxin, Tsh-like haemoglobin protease, antigen 43	Östblom et al. 2011
PAI IV_{536}	Yersiniabactin siderophore system	Östblom et al. 2011
PAI I_{J96}	α-Haemolysin, and P-fimbriae	Schmidt and Hensel. 2004
PAI II_{J96}	α-Haemolysin, Prs fimbriae, cytotoxic, necrotizing factor	Östblom et al. 2011
PAI I_{CFT073}	α-Haemolysin, P-fimbriae and aerobactin	Schmidt and Hensel. 2004
PAI II_{CFT073}	P-fimbriae and iron-regulated genes	Östblom et al. 2011

PAI I_{CFT073}, PAI II_{CFT073}, PAI I_{J96} and PAI II_{J96} was assessed using the previously described primers listed in Table 2 (Sabaté et al. 2006). Template DNA was extracted from *E. coli* isolates by boiling. One isolate from each patient was grown in LB (Luria Bertani Broth, Merck) until the exponential phase with 2 McFarland turbidity. Then, 500 μL of bacterial suspension was centrifuged at 8000 rpm for 5 min and pellet suspended in 200 μL sterile deionized water and boiled at 100 °C for 15 min. After centrifugation at 13000 rpm for 3 min, supernatant was used for PCR. Simplex PCR was performed using DreamTaq PCR Master Mix (Thermo Fisher Scientific), which contains Taq polymerase, dNTPs, $MgCl_2$ and the appropriate buffer. Each PCR tube contained 25 μL reaction mixture composed of 12.5 μL of the master mix, 1.5 μL of each forward and reverse primer solution (in a final concentration of 200 nmol/L), 5 μL of DNA with concentration of 400 ng/μL and nuclease-free water to complete the final volume. PCR was performed using the Gene Atlas 322 system (ASTEC). Amplification involved an initial denaturation at 94 °C, 5 min followed by 35 cycles of denaturation (94 °C, 1 min), annealing (55 °C, 1 min) and extension (72 °C, 1 min), with a final extension step (72 °C, 8 min). The amplified DNA was separated by submarine gel electrophoresis on 1.5 % agarose, stained with ethidium bromide and visualized under UV transillumination. UPEC strains 536 and J96 were used as PAI marker controls.

Statistical analysis The data were analysed with SSPS version 17.0 software (SPSS). A chi-square test was used to determine the statistical significance of the data. A P value of <0.05 was considered significant.

Results

Distribution of PAI markers in commensal and uropathogenic *E. coli* isolates Overall, 192 (96 %) isolates were positive for the presence of PAI markers, 44 commensal isolates (88 %) and 148 UPEC isolates (98.7 %) from patients with symptomatic urinary tract infections.

The frequency of each PAI marker in the patient and control groups is shown in Table 3. Comparison of PAI marker distribution among UPEC and commensal isolates showed that UPEC isolates harboured markers with higher frequency than in commensal isolates except PAI I_{J96} ($P<0.05$). The most prevalent PAI marker among UPEC isolates was PAI IV_{536} (98.7 %), followed by PAI II_{CFT073} (61.3 %), PAI I_{CFT073} (43.3 %), PAI III536 (21.3 %), PAI I_{536} (16.7 %), PAI II_{536} (12 %), PAI II_{J96} (8 %) and PAI I_{J96} (0.7 %). The frequency of PAI IV_{536}, PAI I_{CFT073} and PAI II_{CFT073} in commensal isolates was 84, 6 and 4 %, respectively. PAI III_{536} and PAI II_{J96} were not detected in commensal isolates.

The presence of multiple PAIs with different combinations was found among UPEC isolates. Figure 1 and Table 4 show that many UPEC isolates (79.3 %) carry two or more PAIs compared with commensal isolates (6 %) ($P<0.05$). The number of PAIs per isolate and their specific combinations are shown in Table 4. The mean number of PAIs per isolate was higher in UPEC isolates than commensals ($P<0.05$). The most frequent combinations of PAI markers in UPEC isolates were PAI IV_{536}+PAI II_{CFT073} (18 %) and PAI IV_{536}+PAI I_{CFT073}+PAI II_{CFT073} (18 %), followed by PAI IV_{536}+PAI I_{CFT073} (7.3 %). The most commensal isolates (82 %) had only one PAI marker.

Susceptibility to antimicrobial agents Antimicrobial resistance patterns of isolates are presented in Table 5. In all, 190 (95 %) isolates were resistant to one or more of the 13 tested

Table 2 Primers used in this study

Target	Primer sequence (5′→3′)	Amplicon size (bp)
PAI I_{536}-F	TAA TGC CGG AGA TTC ATT GTC	1800
PAI I_{536}-R	AGG ATT TGT CTC AGG GCT TT	
PAI II_{536}-F	CAT GTC CAA AGC TCG AGC C	1000
PAI II_{536}-R	CTA CGT CAG GCT GGC TTT G	
PAI III_{536}-F	CGG GCA TGC ATC AAT TAT CTT TG	200
PAI III_{536}-R	TGT GTA GAT GCA GTC ACT CCG	
PAI IV_{536}-F	AAG GAT TCG CTG TTA CCG GAC	300
PAI IV_{536}-R	TCG TCG GGC AGC GTT TCT TCT	
PAI I_{CFT073}-F	GGA CAT CCT GTT ACA GCG CGC A	930
PAI I_{CFT073}-R	TCG CCA CCA ATC ACA GCG AAC	
PAI II_{CFT073}-F	ATG GAT GTT GTA TCG C	400
PAI II_{CFT073}-R	ACG AGC ATG TGG ATC TGC	
PAI I_{J96}-F	TCG TGC TCA GGT CCG GAA TTT	400
PAI I_{J96}-R	TGG CAT CCC ACA TTA TCG	
PAI II_{J96}-F	GGA TCC ATG AAA ACA TGG TTA ATG GG	2300
PAI II_{J96}-R	GAT ATT TTT GTT GCC ATT GGT TAC C	

Table 3 Distribution of PAIs among 50 commensal and 150 uropathogenic isolates of *E. coli*

Pathogenicity island	No. (%) of isolates carrying PAIs		*P* value	Total (*n*=200)
	UPEC (*n*=150)	Commensal (*n*=50)		
PAI I_{536}	25 (16.7)	1 (2)	0.007	26 (13)
PAI II_{536}	18 (12)	1 (2)	0.037	19 (9.5)
PAI III_{536}	32 (21.3)	0 (0)	0.000	32 (16)
PAI IV_{536}	148 (98.7)	42 (84)	0.000	190 (95)
PAI I_{CFT073}	65 (43.3)	3 (6)	0.000	68 (34)
PAI II_{CFT073}	92 (61.3)	2 (4)	0.000	94 (47)
PAI I_{J96}	1 (0.7)	1 (2)	0.414	2 (1)
PAI II_{J96}	12 (8)	0 (0)	0.039	12 (6)

A *P* value of <0.05 was considered significant

antimicrobial agents, with the most frequent resistance found against amoxicillin (68 %), amoxicillin/clavulanic acid (55 %), aztreonam (50 %), trimethoprim/sulfamethoxazole (46 %) and tetracycline (43.5 %). Imipenem showed the highest activity against isolates and only 2 % of isolates were imipenem resistant. Prevalence of antibiotic resistance varied among UPEC and commensal isolates, and the frequency of resistance to amikacine, tetracycline, cefepime and ciprofloxacin in UPEC isolates was significantly higher than commensals ($P<0.05$). Antimicrobial drug resistance among UPEC isolates carrying different PAI combinations is presented in Table 6. A total of 132 (66 %) isolates were resistant to at least three different classes of antimicrobial agents and considered as multidrug resistance (MDR): 110 of the UPEC isolates (73.3 %) and 22 of the commensal isolates (44 %) ($P<0.05$). The most prevalent MDR pattern was resistance to β-lactams, tetracycline, gentamicin and trimethoprim/sulfamethoxazole. All MDR isolates of UPEC and commensal were positive for the presence at least one PAI marker. One hundred two (92.7 %) multidrug-resistant UPEC isolates carry two or more PAI markers (Table 7).

Discussion

Horizontal gene transfer (HGT) seems to be an important mechanism for bacterial evolution, let alone genome complexity and plasticity. PAIs, which are large genomic segments and most likely transferred by HGT, contribute to the virulence and survival of the hosting bacterial strain in a particular environment (Middendorf et al. 2004; Gal-Mor and Finlay 2006; Che et al. 2014). PAIs have been studied widely in the genomes of pathogenic bacteria, but little attention has been given to PAIs in the genomes of commensal members of a species (Sabaté et al. 2006). In our study, the presence of eight PAI markers was detected among UPEC and commensal *E. coli* isolates from the stools of healthy subjects. PAI markers were detected in substantial percentage of commensal (88 %) and UPEC isolates (98.6 %) ($P>0.05$). According to our results and previous studies, some isolates of *E. coli* from the intestinal tract of healthy people can be considered potentially virulent, as some isolates showed two PAI markers. Already, it has been reported that ExPEC can asymptomatically colonize the intestinal tract (Koga et al. 2014).

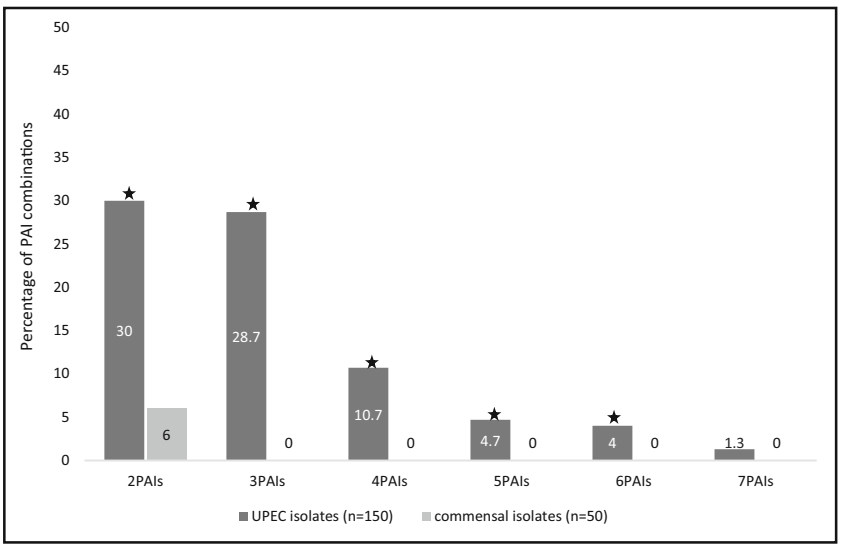

Fig 1 Prevalence of PAI combinations among the 122 *Escherichia coli* isolates (3 commensal and 119 uropathogenic) carrying more than one PAI

Table 4 Specific PAI combinations among the 122 *Escherichia coli* isolates (3 commensal and 119 uropathogenic) carrying more than one PAI

No. of PAIs	PAI combinations	No. (%) of PAIs in UPEC (n=150)	No. (%) of PAIs in commensal (n=50)	Total no. (%) of (n=200)
2 PAIs	PAI IV_{536}+PAI II_{CFT073}	27 (18)	1 (2)	48 (24)
	PAI III_{536}+PAI IV_{536}	4 (2.7)	–	
	PAI IV_{536}+PAI I_{CFT073}	11 (7.3)	2 (4)	
	PAI I_{536}+PAI IV_{536}	2 (1.3)	–	
	PAI IV_{536}+PAI I_{J96}	1 (0.7)	–	
3 PAIs	PAI IV_{536}+PAI I_{CFT073}+PAI II_{CFT073}	27 (18)	–	43 (21.5)
	PAI I_{536}+PAI IV_{536}+PAI II_{J96}	2 (1.3)	–	
	PAI III_{536}+PAI IV_{536}+PAI II_{CFT073}	8 (5.3)	–	
	PAI II_{536}+PAI IV_{536}+PAI II_{CFT073}	2 (1.3)	–	
	PAI III_{536}+PAI I_{CFT073}+PAI II_{CFT073}	1 (0.7)	–	
	PAI I_{536}+PAI IV_{536}+PAI I_{CFT073}	1 (0.7)	–	
	PAI I_{536}+PAI IV_{536}+PAI II_{CFT073}	1 (0.7)	–	
	PAI I_{536}+PAI III_{536}+PAI IV_{536}	1 (0.7)	–	
4 PAIs	PAI I_{536}+PAI IV_{536}+PAI I_{CFT073}+PAI II_{J96}	2 (1.3)	–	16 (8)
	PAI I_{536}+PAI IV_{536}+PAI I_{CFT073}+PAI II_{CFT073}	1 (0.7)	–	
	PAI I_{536}+PAI II_{536}+PAI I_{CFT073}+PAI II_{J96}	1 (0.7)	–	
	PAI II_{536}+PAI IV_{536}+PAI I_{CFT073}+PAI II_{CFT073}	1 (0.7)	–	
	PAI III_{536}+PAI IV_{536}+PAI I_{CFT073}+PAI II_{CFT073}	8 (5.3)	–	
	PAI I_{536}+PAI III_{536}+PAI IV_{536}+PAI I_{CFT073}	1 (0.7)	–	
	PAI I_{536}+PAI III_{536}+PAI IV_{536}+PAI II_{CFT073}	1 (0.7)	–	
	PAI II_{536}+PAI III_{536}+PAI IV_{536}+PAI II_{CFT073}	1 (0.7)	–	
5 PAIs	PAI I_{536}+PAI II_{536}+PAI IV_{536}+PAI I_{CFT073}+ PAI II_{CFT073}	5 (3.3)	–	7 (3.5)
	PAI II_{536}+PAI III_{536}+PAI IV_{536}+PAI I_{CFT073}+ PAI II_{CFT073}	1 (0.7)	–	
	PAI I_{536}+PAI III_{536}+PAI IV_{536}+PAI II_{CFT073}+ PAI II_{J96}	1 (0.7)	–	
6 PAIs	PAI I_{536}+PAI II_{536}+PAI IV_{536}+PAI I_{CFT073}+ PAI II_{CFT073}+PAI II_{J96}	3 (2)	–	6 (3)
	PAI I_{536}+PAI II_{536}+PAI III_{536}+PAI IV_{536}+ PAI II_{CFT073}+PAI II_{J96}	2 (1.3)	–	
	PAI I_{536}+PAI II_{536}+PAI III_{536}+PAI IV_{536}+ PAI I_{CFT073}+PAI II_{CFT073}	1 (0.7)	–	
7 PAIs	PAI I_{536}+PAI II_{536}+PAI III_{536}+PAI IV_{536}+ PAI I_{CFT073}+PAI II_{CFT073}+PAI II_{J96}	2 (1.3)	–	2 (1)

Furthermore, there was evidence that the intestinal niche may harbour *E. coli* isolates, with a large number of accumulated PAIs (Sabaté et al. 2006). However, in our study, commensal isolates contained fewer PAI combinations than UPEC isolates. Previous studies showed that UPEC isolates harboured PAI markers with significantly higher frequency than commensals (Sabaté et al. 2006; Navidinia et al. 2013a). Distribution of various PAIs in our study showed the same pattern with other studies (Sabaté et al. 2006; Li et al. 2010; Navidinia et al. 2013b). PAI IV_{536}, also termed high-pathogenicity island (HPI), was found most frequently in both commensal and UPEC isolates and is reported to be the most ubiquitous PAI found in Enterobacteriaceae. In previous studies (Sabaté et al. 2006; Navidinia et al. 2013b), PAI IV_{536} was detected in 38 and 18 % of faecal *E. coli* isolates respectively, compared with 84 % of isolates in the present study. The high frequency of PAI IV_{536} in commensal isolates has led to the suggestion that HPI may be a fitness island rather than a pathogenicity island (Sabaté et al. 2006). Previous studies (Middendorf et al. 2004) demonstrated that PAI IV is stable in *E. coli* 536, a fact that could explain its high frequency. The remaining PAI markers except PAI I_{J96} had a similar distribution in commensal and UPEC isolates but with significantly higher frequency in UPEC isolates. Similar to our results, it was suggested that PAI I_{J96} might not be important in the pathogenesis of urosepsis (Sabaté et al. 2006).

Table 5 Prevalence of antibiotic resistance among 200 uropathogenic and commensal *E. coli* isolates

Antimicrobial agents	No. (%) of resistant commensal *E. coli* (*n*=50)	No. (%) of resistant uropathogenic *E. coli* (*n*=150)	*P* value	No. (%) of total resistant isolates (*n*=200)
Amoxicillin	31 (62)	105 (70)	0.29	136 (68)
Cefoxitine	8 (16)	21 (14)	0.64	29 (14.5)
Ceftazidime	12 (24)	57 (38)	0.07	69 (34.5)
Cefotaxime	15 (30)	56 (37.3)	0.35	71 (35.5)
Cefepime	5 (10)	48 (32)	0.002	53 (26.5)
Amoxicillin/clavulanic acid	25 (50)	85 (56.6)	0.41	110 (55)
Imipenem	0	4 (2.7)	0.24	4 (2.7)
Azteronam	22 (44)	78 (52)	0.33	100 (50)
Gentamicin	12 (24)	50 (33.4)	0.21	62 (31)
Tetracycline	13 (26)	74 (49.3)	0.004	87 (43.5)
Trimethoprim/sulfamethoxazole	20 (40)	72 (48)	0.32	92 (46)
Amikacin	5 (10)	35 (23.3)	0.04	40 (20)
Ciprofloxacin	8 (16)	47 (31.4)	0.03	55 (27.5)

% shown for UPEC or commensal isolates is % compared to the total number of UPEC or commensals. *P* value of <0.05 was considered significant

Several different combinations of PAI markers were found among 79.3 % of UPEC isolates, while 6 % of commensals had two PAI markers. Our results showed that the mean number of PAIs per isolate was higher among UPEC in comparison with commensals ($P<0.05$).

Antimicrobial resistance and the spread of resistance genes among pathogenic *E. coli* isolates have become a major public health problem in developing countries (Su et al. 2006). Treatment of infections associated with multidrug-resistant *E. coli* is further complicated in Asian countries such as Taiwan, India and Iran (Rice 2009; Phongpaichit et al. 2011). In our study, 95 % of *E. coli* isolates were resistant to one or more antimicrobial agents and 66 % were multidrug resistant. High frequency of antibiotic resistance among UPEC isolates was reported in previous studies in Iran (Farshad et al. 2012; Rezaee et al. 2011; Neamati et al. 2015). The high incidence of amoxicillin (68 %) and amoxicillin/clavulanic acid (55 %) resistance in the present study is most probably due to the widespread use of these antimicrobial agents in our country. Furthermore, the loss and gain of resistance genes by mobile genetic elements are an important mechanism in the development of multidrug-

Table 6 Distribution of PAI combinations among resistant UPEC isolates

Antimicrobial agents	No. (%) of resistance among 148 UPEC isolates carrying PAI combinations							Total no. of resistant UPEC
	1 PAI (*n*=29)	2 PAIs (*n*=45)	3 PAIs (*n*=43)	4 PAIs (*n*=16)	5 PAIs (*n*=7)	6 PAIs (*n*=6)	7 PAIs (*n*=2)	
Amoxicillin	15 (51.7)	33 (73.3)	31 (72.1)	14 (87.5)	5 (71.4)	5 (83.3)	2 (100)	105
Cefoxitine	9 (31)	5 (11.1)	5 (11.6)	2 (12.5)	0 (0)	0 (0)	0 (0)	21
Ceftazidime	6 (20.7)	15 (33.3)	23 (53.5)	4 (25)	4 (57.1)	4 (66.7)	1 (50)	57
Cefotaxime	6 (20.7)	14 (31.1)	21 (48.8)	6 (37.5)	4 (57.1)	5 (83.3)	0 (0)	56
Cefepime	8 (27.6)	12 (26.7)	19 (44.2)	3 (18.8)	3 (42.9)	3 (50)	0 (0)	48
Amoxicillin/clavulanic acid	10 (34.5)	31 (68.9)	25 (58.1)	8 (50)	6 (85.7)	3 (50)	2 (100)	85
Imipenem	2 (6.9)	0 (0)	2 (4.7)	0 (0)	0 (0)	0 (0)	0 (0)	4
Azteronam	8 (27.6)	24 (53.3)	26 (60.5)	9 (56.3)	5 (71.4)	5 (83.3)	1 (50)	78
Gentamicin	7 (24.1)	10 (22.2)	18 (41.9)	6 (37.5)	5 (71.4)	3 (50)	1 (50)	50
Tetracycline	17 (58.6)	23 (51.1)	22 (51.2)	7 (43.8)	2 (28.6)	2 (33.3)	1 (50)	74
Trimethoprim/sulfamethoxazole	14 (48.3)	22 (48.9)	23 (53.5)	6 (37.5)	4 (57.1)	1 (16.7)	2 (100)	72
Amikacin	19 (65.5)	10 (22.2)	3 (7)	1 (6.3)	1 (14.3)	1 (16.7)	0 (0)	35
Ciprofloxacin	6 (20.7)	5 (11.1)	19 (44.2)	8 (50)	5 (71.4)	3 (50)	1 (50)	47

% shown for each antibiotic is % compared to total no. of each PAI combination

Table 7 No. (%) of multidrug resistance (MDR) UPEC isolates according to PAI combinations % shown for multidrug resistance UPEC isolates is % compared to total no. of each PAI combination

PAIs no.	MDR no. (%)
1 PAI (n=29)	8 (27.6)
2 PAI (n=45)	40 (88.9)
3 PAI (n=43)	35 (81.4)
4 PAI (n=16)	16 (100)
5 PAI (n=7)	5 (71.4)
6 PAI (n=6)	4 (66.7)
7 PAI (n=2)	2 (100)
Total (n=148)	110

resistant isolates (Wozniak and Waldor 2010). Similar to our study, Navidinia et al. (2013a) and Neamati et al. (2015) were reported 78 and 51.4 % resistance to aztreonam. High-level resistance to aztreonam was reported by Pobiega et al. (2013) among the extended-spectrum β-lactamases (ESBL)-producing *E. coli* isolates. In a study carried out in Tamilnadu, India, the majority of UPEC isolates from UTI patients exhibited MDR phenotype including amoxicillin (96.2 %), tetracycline (72.7 %), cefotaxime (74.8 %) and ciprofloxacin (70.4 %) resistance (Murugan et al. 2012). According to our results, imipenem (98 %) showed the lowest resistance against UPEC and commensal isolates, and only 2 % of isolates were imipenem resistant. In previous studies (Rezaee et al. 2011; Neamati et al. 2015), resistance to imipenem was detected in 1.4 and 0.7 % of UPEC isolates, respectively. According to results, antimicrobial resistance among UPEC isolates was higher than commensal isolates. The high prevalence of antibiotic resistance in UPEC isolates may be due to acquisition of the resistance genes from intestinal microbiota as reservoirs for transmission of these genes (Ravi et al. 2014).

Ethical considerations

This study was performed using routine samples obtained from patients admitted to hospitals, and thus, ethical approval was not required.

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