TÜBİTAK

Original Article

Turk J Med Sci
2012; 42 (Sup.1): 1328-1337
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E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-1201-31

Characterization of uropathogenic *Escherichia coli* strains obtained from urology outpatient clinic of Ege Medical Faculty in İzmir

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Aim: To identify the *papG* gene and its allelic variation in uropathogenic *Escherichia coli* (UPEC) strains isolated from patients with acute pyelonephritis and cystitis.

Materials and methods: Seventy-five *E. coli* strains isolated from patients admitted to the University of Ege Medical Faculty urology outpatient clinic were isolated and identified phenotypically. All of these strains were examined for the *papG* gene and allelic distribution of this gene with the multiplex polymerase chain reaction technique.

Results: *papG* genes were found in 24 of 75 *E. coli* strains. Of these 24 strains, 7 (29%) had *papG* class II only, 8 (33%) had class III only, and 9 (38%) had both class II and III. Phylogenetically, it was found that 31 belonged to group B2, 19 to group D, 20 to group A, and 5 to group B1. Serotyping was performed and the positivity was found to be 39%. When the antibiotic resistance profiles of the 75 strains were evaluated, 41 (55%) of them were found to be resistant to ampicillin, 35 (47%) to ciprofloxacin, and 35 (47%) to trimethoprim/sulfamethoxazole. In addition, 23 strains (31%) produced extended-spectrum beta-lactamase.

Conclusion: In this study, the rate of *papG*-positive strains was found to be low. However, there is no consensus on the molecular definition of UPEC. Although the presence of the *papG* gene indicates that the strains are UPEC, absence of the *papG* gene does not suggest that the strains are not UPEC.

Key words: UPEC, *E. coli*, *papG*, urinary tract infections, phylogenetic grouping

Introduction

Urinary tract infections (UTIs) are among the most common infections that affect humans (1,2). Fifty percent of all women will experience at least 1 UTI in their lifetime and, of those, about 25% will have 1 or more recurrent infections (3,4). In 90% of uncomplicated UTIs, the most common bacterium is *Escherichia coli* (2,5). *E. coli* strains causing disease outside the gastrointestinal tract have been named extraintestinal pathogenic *E. coli* (ExPEC) (6), and among the ExPEC, uropathogenic *E. coli* (UPEC) is the most common pathogen in humans (7). UPEC strains are characterized with specific virulence factors closely related with colonization and persistence of bacteria in the urinary tract. These factors include adhesins or fimbriae, siderophore systems, and toxins. For the initiation of UTI, colonization of UPEC is required. Therefore, colonization and adhesion to host tissues is crucial for UPEC pathogenesis. At this stage, expression of the P-fimbriae frequently present in UPEC, and especially in UPEC strains isolated from patients with pyelonephritis, is extremely important (8,9). So

Received: 13.01.2012 – Accepted: 07.05.2012

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far, P-fimbriae have been detected in the majority of patients with acute pyelonephritis and cystitis who have a normal immune system (10,11). G adhesin, located at the tip of P-fimbriae and encoded by the *pap* operon, contributes to the progress of the disease by binding to specific receptors in the uroepithelial cells (8,12).

Depending on receptor specificity, *papG* adhesin has 3 different variants (Class I to Class III), and in studies conducted to date it has been proposed that there is a relationship between the specific alleles of genes encoding G adhesin and various forms of the disease (10,12,13).

Phylogenetic analyses by multilocus enzyme electrophoresis have shown that *E. coli* strains fall into 4 main phylogenetic groups: A, B1, B2, and D. While nonpathogenic *E. coli* strains are typically from phylogenetic groups A and B1, ExPEC, particularly UPEC strains, frequently are members of group B2 or group D and often exhibit specific O:K:H serotypes (14–16).

The aim of this study was to detect *papG* alleles and to define the distribution of 3 *papG* alleles among *E. coli* isolates from 75 patients with acute cystitis and pyelonephritis at a hospital in İzmir, and to investigate the link between phylogeny and virulence factors.

Materials and methods

Bacterial strains

Seventy-five *E. coli* strains were isolated from the urine cultures of patients with acute cystitis and acute pyelonephritis that presented to the University of Ege Medical Faculty urology outpatient clinic between January and April 2010. Identification of these strains was performed biochemically with the VITEK 2 Compact System (bioMérieux, France) in the hospital. Reference *E. coli* strains J96 (*papG*I and III), 2H16 (*papG* II), K12 (negative control for *papG*, positive control for *uidA*), EcoR20 (A), EcoR48 (D), EcoR58 (B1), and EcoR62 (B2) were kindly provided by J. R. Johnson (University of Minnesota) and used as control strains. *E. coli* ATCC 25922 was used as a quality control strain for antibiotic susceptibility testing. Strains were stored at –30 °C in tryptic soy broth with 20% glycerol until they were used.

The ethical committee approval date was 16.11.2009 and the decision number was 09/11.1/10.

Serotyping

Isolated *E. coli* strains were serotyped with polyvalent antisera (O Pool 8 ExPEC, Statens Serum Institut SSI Diagnostica, Denmark) for ExPEC strains including O1, O2, O4, O6, O7, O15, O18ac, and O75 serotypes. The assay was performed according to the manufacturer's instructions. *E. coli* strain J96 serotype O4:K6 was used for positive control.

Antimicrobial susceptibility testing

In vitro susceptibility of *E. coli* strains was determined with the disk diffusion method according to Clinical and Laboratory Standards Institute guidelines (17), and then the following drugs were tested: cephalothin (30 mg), imipenem (10 mg), cefotaxime (30 mg), cefepime (30 mg), ciprofloxacin (5 mg), ampicillin (10 mg), aztreonam (30 mg), ceftazidime (30 mg), amikacin (30 mg), meropenem (10 mg), ampicillin/sulbactam (20 mg), cefuroxime (30 mg), piperacillin/tazobactam (110 mg), gentamicin (10 mg), amoxicillin/clavulanic acid (30 mg), and trimethoprim/sulfamethoxazole (25 mg) (Oxoid, UK). Extended-spectrum beta-lactamase (ESBL) production was detected using the double-disk synergy (DDS) test in our laboratory (18). Similarly, the antimicrobial susceptibility test and ESBL production were performed with the VITEK 2 Compact System in the hospital.

DNA extraction, *uidA* gene detection, and phylogenetic analysis

DNA extraction was carried out with the rapid minipreparation procedure (19). The 75 *E. coli* isolates were tested for the *uidA* gene with appropriate primers by conventional polymerase chain reaction (PCR) (J. R. Johnson, 2010, personal communication). Phylogenetic grouping of all isolates was determined by a modified version of the multiplex PCR method as described previously (15). As distinct from the method used by Clermont et al. (15), we used different TSPE4C.2 primers (20). The primer sequences, the size of the amplified fragment (base pair), and the annealing temperature are shown in Table 1. The conventional and multiplex PCRs were performed using the NanoHelix PCR kit (HelixAmp™Taq DNA polymerase, NanoHelix Co.,

Table 1. List of the primers used in the study.

Genes	Primer sequence (5'-3')	Primer name	Size of product (bp)	Source of primer
UidA	F GCGTCTGTTGACTGG CAG GTGGTG G	*uidA*F	508 bp	Johnson, PC, 2010
	R GTTGCCCGCTTCGAAACCAATGCC T	*uidA*R		
papG I	F CTGTAATTACGGAAGTGATTTCTG	pGf	1190 bp	21
	R TCC AGA AATAGCTCATGTAACCCG	PG 1r		
papG II and III	F CTGTAATTACGGAAGTGATTTCTG	pGf	1070 bp	21
	R ACT ATCCGG CTC CGG ATA AAC CAT	pGr		
papG class I	F CAACCTGCT CTC AATCTTTAC TG	PF1	692 bp	10
	R CAT GGCTGGTTGTTC CTA AAC AT	PF1,3		
papG class II	F GGAATGTGGTGATTA CTC AAA GG	PF2	562 bp	10
	R TCC AGA GACTGTGCAGAAGGA C	PF2,3		
papG class III	F CAT GGCTGGTTGTTC CTA AAC AT	PF1,3	421 bp	10
	R TCC AGA GACTGTGCAGAAGGA C	PF2,3		
chuA	F GACGAACCAACGGTCAGG AT	ChuA.1	279 bp	15
	R TGCCGC CAG TACCAA AGA CA	ChuA.2		
Yja	F TGAAGTGTCAGG AGA CGC TG	YjaA.1	211 bp	15
	R ATG GAG AATGCGTTC CTC AAC	YjaA.2		
TSPE4.C2	F AGTAATGTCGGGGCATTC AG	TSPE4.CII' F	151 bp	20
	R TCGCGCCAACAAAGT ATT ACG	TSPE4.CII'R		

PC: personal communication; F: forward primer; R: reverse primer.

Ltd., South Korea). These assays were carried out according to the manufacturer's instructions.

Detection of the *papG* gene and allelic variation

To investigate the presence of the *papG* gene and its allelic variation, we used flanking and internal allele-specific *papG* primers (Table 1). First, *papG* was detected by multiplex PCR with flanking primers as previously described (21). *papG*-positive strains were then investigated in terms of allelic variation. For this, 3 *papG* classes were determined by multiplex PCR with internal primers (Table 1) as previously described (10), but this method was modified. For this purpose, we used a Hot Start PCR kit (i-StartTaq™ DNA polymerase, iNtRON Biotechnology, South Korea) and the assay was performed in a total volume 20 µL of mixture containing 2 µL PCR buffer, 2 µL dNTP, 0.2 µL DNA polymerase, 2 µL template DNA, 7 pmol PF2 and PF2,3, and 10 pmol PF1 and P1,3. PCR parameters were as follows: 2 min at 94 °C; 30 cycles of 20 s at 94 °C, 10 s at 63 °C, and 40 s at 72 °C; and final extension of 5 min at 72 °C.

Products of conventional and multiplex PCRs were electrophoresed in 1.5% and 2% agarose gel with 0.5 µg/mL ethidium bromide, respectively. Gels were photographed by using an ultraviolet transilluminator and digital capture system (DNr Bio-Imaging Systems Ltd., Israel). The size of amplicons was determined by comparing them with a 100-bp DNA ladder (ABM Inc., Canada).

Statistical analysis

The associations between phylogeny and antimicrobial susceptibility results were tested using Fisher's exact test (2-tailed). SPSS 11.0 for Windows (SPSS Inc., USA) was used in the study. $\alpha = 0.05$ was used for all analysis.

Results

Presence of *uidA* gene, serotyping, and phylogenetic analysis

The 75 *E. coli* isolates were categorized for the presence of the *uidA* gene, serogroup, and phylogenetic status as listed in Table 2; 73 (97%) of the 75 *E. coli* strains were found positive for the *uidA* gene (Figure 1).

Our strains were tested with polyvalent antisera, and of the 75 strains, 29 (39%) were serotyped as positive and 46 (61%) were negative.

Phylogenetic grouping was determined as described by Clermont et al. (15) and was as follows: 31 (41%) strains belonged to group B2, 19 (25%) belonged to group D, 20 (27%) belonged to A, and 5 (7%) belonged to B1 (Figure 2).

Table 2. List of 75 *E. coli* isolates with clinical data, serotyping, *uidA* gene, and phylogenetic status.

Identification code	Serotyping	Clinical data	*uidA* gene	Phylogenetic status	Identification code	Serotyping	Clinical data	*uidA* gene	Phylogenetic status
1	+	Pyelonephritis	+	D	39	-	Pyelonephritis	+	B2
2	+	Cystitis	+	B2	40	-	Cystitis	+	B2
3	+	Cystitis	+	B2	41	-	Pyelonephritis	+	B2
4	-	Pyelonephritis	+	B2	42	-	Cystitis	+	B2
5	-	Pyelonephritis	+	A	43	-	Cystitis	+	A
6	-	Pyelonephritis	+	A	44	-	Pyelonephritis	+	B2
7	-	Pyelonephritis	+	A	45	+	Cystitis	+	D
8	-	Cystitis	+	A	46	-	Cystitis	+	D
9	-	Cystitis	+	D	47	-	Cystitis	+	A
10	-	Cystitis	+	A	48	-	Pyelonephritis	+	A
11	-	Cystitis	+	A	49	-	Pyelonephritis	+	D
12	+	Pyelonephritis	+	B1	50	+	Pyelonephritis	+	B2
13	-	Cystitis	+	D	51	-	Pyelonephritis	+	A
14	-	Cystitis	+	A	52	-	Cystitis	+	A
15	+	Pyelonephritis	+	A	53	+	Pyelonephritis	+	B2
16	+	Pyelonephritis	+	B2	54	-	Pyelonephritis	+	B2
17	+	Pyelonephritis	+	B2	55	+	Pyelonephritis	+	A
18	-	Cystitis	+	B1	56	+	Cystitis	+	B2
19	+	Pyelonephritis	+	D	57	+	Cystitis	+	B2
20	-	Cystitis	+	B2	58	-	Cystitis	+	D
21	+	Pyelonephritis	+	B2	59	-	Pyelonephritis	+	B1
22	+	Cystitis	+	D	60	-	Cystitis	+	D
23	+	Cystitis	+	D	61	-	Pyelonephritis	+	D
24	+	Cystitis	+	B2	62	+	Cystitis	+	B2
25	+	Pyelonephritis	+	B1	63	-	Pyelonephritis	+	A
26	+	Cystitis	+	D	64	-	Cystitis	+	A
27	+	Cystitis	-	B2	65	-	Cystitis	+	D
28	+	Cystitis	+	B2	66	-	Cystitis	+	B1
29	+	Pyelonephritis	+	B2	67	-	Pyelonephritis	+	A
30	-	Cystitis	+	D	68	+	Cystitis	+	D
31	-	Cystitis	-	B2	69	-	Pyelonephritis	+	B2
32	-	Cystitis	+	A	70	-	Cystitis	+	B2
33	-	Cystitis	+	D	71	-	Cystitis	+	B2
34	+	Cystitis	+	B2	72	-	Pyelonephritis	+	B2
35	+	Pyelonephritis	+	D	73	-	Cystitis	+	A
36	+	Cystitis	+	A	74	+	Pyelonephritis	+	B2
37	-	Pyelonephritis	+	B2	75	-	Cystitis	+	B2
38	-	Pyelonephritis	+	D					

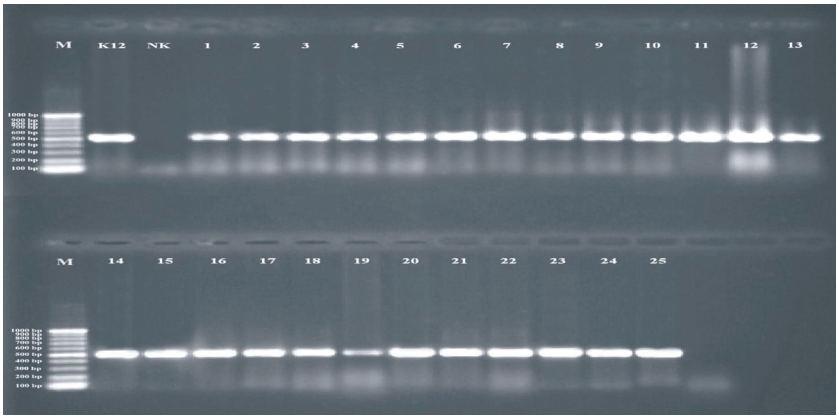

Figure 1. Presence of the *uidA* gene in 25 *E. coli* strains. Line 1: marker (M); line 2: positive control (K12); line 3: negative control (NK); lines 4–16: *E. coli* strains; line 17: marker (M); lines 18-29: *E. coli* strains (data for other strains not shown).

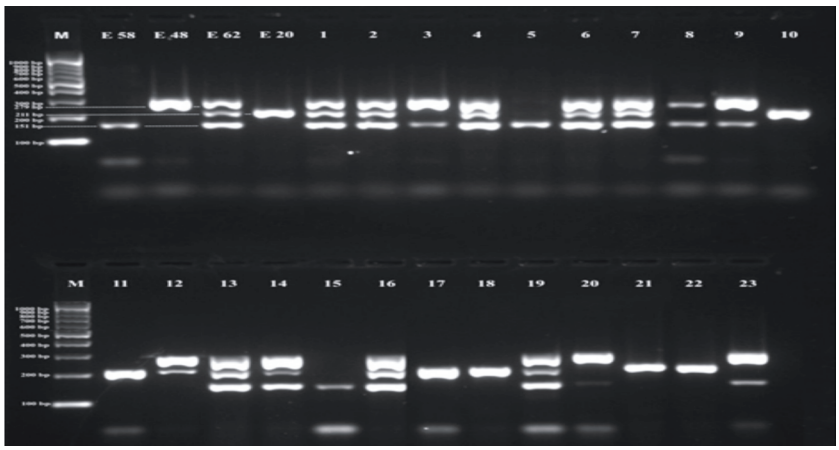

Figure 2. Phylogenetic distribution of 23 *E. coli* isolates. Line 1: marker (M); line 2: E58; line 3: E48; line 4: E62; line 5: E20; lines 6–15: *E. coli* isolates (strains numbered as 17, 21, 23, 24, 25, 27, 29, 35, 38, and 67); line 16: marker (M); lines 17-29: *E. coli* strains (strains numbered as 43, 50, 53, 54, 12, 2, 11, 7, 3, 9, 36, 52, and 30) (data for other strains not shown).

Antimicrobial resistance

Of the 75 isolates, 41 (55%) were resistant to ampicillin, 35 (47%) to ciprofloxacin, and 35 (47%) to trimethoprim/sulfamethoxazole. In addition, 23 isolates (31%) were found to produce ESBL (Table 3).

Detection and distribution of *papG* alleles and clinical correlation

papG was detected by PCR in 24 (32%) of the 75 strains (Figure 3). Seven (29%) strains had *papG* class II only, 8 (33%) strains had class III only, and 9 (38%) strains had both class II and III (Figure 4). When the relationship between *papG* alleles and the diagnosis was inspected, it was found that whereas of the 15 patients with pyelonephritis, 5 had *papG* II, 5 had *papG* III, and 5 had *pap G II* and III together, of the 9 patients with cystitis, 2 had *papG* II, 3 had *papG* III, and 4 had *papG* II and III together (Table 4).

Association between phylogeny and antimicrobial susceptibility

When the relationship between the phylogenetic distribution of strains and ciprofloxacin and TMP-SMX (which are widely used in treatment) was inspected, most of the ciprofloxacin resistance (65%) was observed in group A strains and TMP-SMX resistance (63%) in group D strains, and these results were not statistically significant.

Table 3. Antibiotic resistance of *E. coli* isolates.

Antibiotic	Resistant (n (%)) (n = 75)
Ampicillin	41 (55)
Gentamicin	26 (35)
Amikacin	0
Amoxicillin/clavulanic acid	18 (24)
Ampicillin/sulbactam	16 (21)
Piperacillin/tazobactam	11 (15)
Cephalothin	27 (36)
Cefepime	22 (30)
Ceftriaxone	23 (31)
Ceftazidime	22 (29)
Cefuroxime	24 (32)
Ciprofloxacin	35 (47)
Trimethoprim/sulfamethoxazole	35 (47)
Imipenem	0
Meropenem	0
Aztreonam	25 (33)
ESBL	23 (31)

n: isolate number.

Of the 23 ESBL-positive strains, 12 (52%) belonged to group B2, 7 (31%) to group A, and 4 (17%) to group D. ESBL production was not observed in group B1 at all.

Discussion

In this study, 75 *E. coli* strains that lead to UTIs were isolated from patients with pyelonephritis and cystitis. Because the *uidA* gene is present in more than 90% of *E. coli* strains (22,23), the presence of the *uidA* gene was investigated as an identification marker. The *uidA* gene was detected in 97% of our isolates.

Strains isolated in this study were tested with O pool 8 ExPEC polyvalent antisera. O antigens generally seen in UPEC strains usually include O1, O2, O4, O6, O7, O8, O15, O16, O18ac, O25, O50, and O75 (24,25), but the antisera used in this study did not include O8, O16, O25, or O50. In addition, strains were not tested with monovalent antisera specific to UPEC. Hence, the rate of positivity was 39%, and it was thought that this low positivity rate was due to the fact that the polyvalent did not include certain O antigens specific to UPEC strains, or that strains included serological phenotypes specific to other pathotypes.

In phylogenetic analyses performed to date, it has been reported that ExPEC strains mostly belong to group B2, followed by group D (15,16). As expected, in our study, too, the most common phylogenetic group in the UPEC isolates was B2.

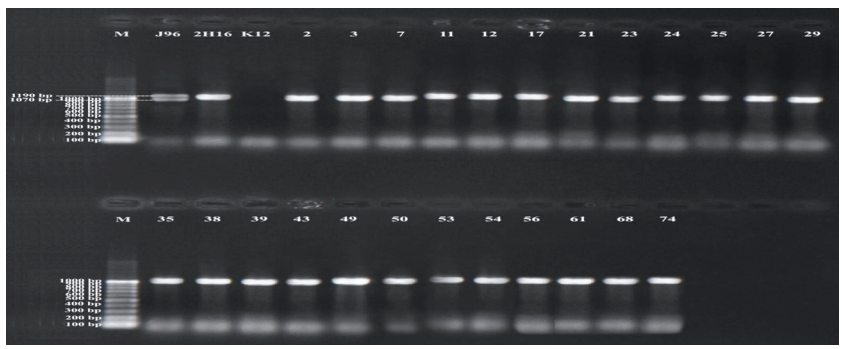

Figure 3. Detection of the *papG* gene. Line 1: marker (M); line 2: J96 (positive control for *papG* I and III); line 3: 2H16 (positive control for *papG* II); line 4: K12 (negative control for *papG*); line 5–16: *papG*-positive strains; line 17: marker (M); lines 18-29: *papG*-positive isolates.

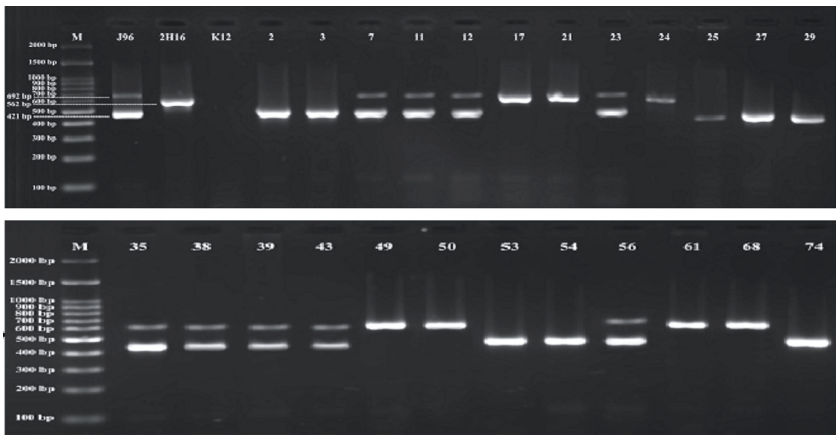

Figure 4. Determination of *papG* alleles. A) Line 1: marker (M); line 2: J96 (positive control for *papG* I and III); line 3: 2H16 (positive control for *papG* II); line 4: K12 (negative control for *papG*); lines 5–16: distribution of *papG* alleles in *papG*-positive strains. B) Line 1: marker (M); lines 2–13: distribution of *papG* alleles in *papG*-positive strains.

Table 4. Distribution of *papG* allele in *papG*-positive *E. coli* strains, clinical data, and phylogenetic status.

Identification code	Clinical data	*papG* class I	*papG* class II	*papG* class III	*papG* class II/III	Phylogenetic status
2	Cystitis	–	–	+	–	B2
3	Cystitis	–	–	+	–	B2
7	Pyelonephritis	–	–	–	+	A
11	Cystitis	–	–	–	+	A
12	Pyelonephritis	–	–	–	+	B1
17	Pyelonephritis	–	+	–	–	B2
21	Pyelonephritis	–	+	–	–	B2
23	Cystitis	–	–	–	+	D
24	Cystitis	–	+	–	–	B2
25	Pyelonephritis	–	–	+	–	B1
27	Cystitis	–	–	+	–	B2
29	Pyelonephritis	–	–	+	–	B2
35	Pyelonephritis	–	–	–	+	D
38	Pyelonephritis	–	–	–	+	D
39	Pyelonephritis	–	–	–	+	B2
43	Cystitis	–	–	–	+	A
49	Pyelonephritis	–	+	–	–	D
50	Pyelonephritis	–	+	–	–	B2
53	Pyelonephritis	–	–	+	–	B2
54	Pyelonephritis	–	–	+	–	B2
56	Cystitis	–	–	–	+	B2
61	Pyelonephritis	–	+	–	–	D
68	Cystitis	–	+	–	–	D
74	Pyelonephritis	–	–	+	–	B2

In studies performed to date, P-fimbriae have been determined in *E. coli* strains isolated from the majority of patients with UTI and urosepsis (1,10,11,13,26). While the *papG* positivity rate was 76% in a study of urosepsis patients (13) and 65% in another study of patients with pyelonephritis and bacteremia (26), in our study, the *papG* gene was identified in 24 (32%) of the 75 *E. coli* strains isolated from 42 patients with cystitis and 33 with pyelonephritis.

The fact that the *papG* gene was not detected in the majority of the strains in this study does suggest that these strains are not UPEC strains (Johnson, 2010, personal communication). Hence, the presence of the *papG* gene alone cannot be regarded as an adequate marker for the identification of UPEC at the molecular level. The presence of this gene can provide information about the level of infection if it is associated with pyelonephritis. Therefore, other virulence genes should be investigated for extensive epidemiological studies.

When the allelic distribution of *papG* is considered, only 7 strains had *papG* II and 8 strains had *papG* III only, but *papG* II/III was observed at a higher rate. In addition, when the relationship between the diagnosis and *papG* alleles detected is considered, no definite correlation was observed. In other studies (10,13,26), while the *papG* II/III combination of alleles was rarely observed, in our study it was detected at a higher rate in patients with cystitis and pyelonephritis. This is thought to result from the diagnoses of urine samples of the patients or from regional differences.

Picard et al. stated that there was a link between virulence factors of phylogeny and extraintestinal *E. coli* strains (14). According to their findings, the *pap* operon was found in the strains of phylogenetic groups D and B1, and most frequently in group B2. Our results support these findings.

It is known that antibiotic susceptibility phenotypes vary from one country to another or even from one region to another. Indeed, in a study conducted by İnan and Gürler in Turkey, ampicillin resistance was determined in 68%, TMP-SMX resistance in 67%, and amoxicillin-clavulanic acid resistance in 38% of the patients who presented to outpatient clinics (27). In another study, it was reported that of the *E. coli* strains isolated from patients in the outpatient clinic, 79% were resistant to ampicillin, 45% to TMP-SMX, and 23% to ciprofloxacin (28). In our study, the resistance rates to TMP-SMX and ciprofloxacin, antibiotics widely used in empirical treatment, were both 47%.

When the resistance rates to ampicillin, trimethoprim/sulfamethoxazole, and ciprofloxacin, which are the oral options for treatment, are considered, ampicillin should not be used for empiric treatment since the resistance rate to ampicillin is above 50%. In addition, in Turkey, resistance to TMP-SMX and ciprofloxacin has increased steadily as antibiotics are widely used in treatment. Therefore, today it is extremely important to conduct new research on antibiotics and to detect the relationship between bacterial virulence factors and the results obtained from these studies.

In many studies conducted to date investigating the relationship between antibiotics such as quinolones (nalidixic acid, pipemidic acid), fluoroquinolones (ciprofloxacin, norfloxacin), trimethoprim/sulfamethoxazole, beta-lactams (ampicillin), extended spectrum cephalosporins, and cephamycin and bacterial virulence factors and phylogenetic groups, it has been reported that organisms resistant to these antibiotics belong to non-B2 groups and contain virulence genes at a lower rate (29–31). Relatedly, in this study, when the relationship between *papG* and ciprofloxacin and trimethoprim/sulfamethoxazole resistance was considered, it was observed that of the 24 *papG* positive strains, 6 developed resistance to ciprofloxacin and 8 to TMP-SMX. In the relationship between the phylogenetic groups to which the phylogenetically grouped 75 *E. coli* strains belong and resistance to ciprofloxacin and TMP-SMX, no statistically significant correlation was detected. However, the high resistance to TMP-SMX observed in strains that phylogenetically belong to group D suggests that these strains clonally belong to group A or O15: K52: H1. In order to reach a definitive diagnosis, additional tests are needed (Johnson, 2012, personal communication). In our study, in addition to these relationships, the relationship between ESBL production and *papG* was evaluated, and the *papG* gene was identified only in 5 (22%) of the 23 strains that can produce ESBL. These results support the argument that strains whose antibiotic resistance is high have low virulence

factors. However, in this study, it was observed that 52% of the ESBL-producing strains belonged to group B2. It is thought that ESBL strains belonging to this group might be ST131 (Johnson, 2012, personal communication).

There is a continuous fight between pathogenic microorganisms and host cells in terms of attack and defense mechanisms. In this fight, pathogenic microorganisms defeat the host cells due to their virulence factors and rapid evolution, and they develop resistance to all kinds of treatment methods. As can be seen in previous studies, since strains of pathogenic microorganisms continuously acquire new genes via horizontal gene transfer, continuous changes occur in genes special to pathotypes. Therefore, it is becoming more and more difficult to reach a definitive diagnosis in distinguishing pathotypes. The results of our research revealed that there are not many studies conducted in Turkey on *E. coli* pathotypes and uropathogenic *E. coli*. Studies regarding these issues have usually focused on antibiotic resistance. Therefore, in Turkey, detailed studies should be performed especially on *E. coli* pathotypes, virulence genes special to pathotypes should be investigated, and comprehensive research focusing on the production of new medicines and vaccines that can overcome these virulence mechanisms should be conducted.

Acknowledgments

This study was supported by the Ege University Scientific Research Project (BAP), 2006-FEN-068. We would like to extend our thanks to Prof Dr James R. Johnson, who provided us with type species; to Prof Dr Oktay Nazlı, who helped us to associate urine samples with the diagnoses; and to Dr Hayal Boyacıoğlu, who performed the statistical analysis.

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