Distribution of phylogenetic groups, adhesin genes, biofilm formation, and antimicrobial resistance of uropathogenic *Escherichia coli* isolated from hospitalized patients in Thailand

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Background: Urinary tract infections (UTIs) are the most common bacterial infections and are often caused by uropathogenic *Escherichia coli* (UPEC). We investigated the distribution of phylogenetic groups, adhesin genes, antimicrobial resistance, and biofilm formation in *E. coli* isolated from patients with UTIs.

Methods: In the present study, 208 UPEC isolated from Thai patients were classified into phylogenetic groups and adhesin genes were detected using multiplex PCR. Antimicrobial susceptibility testing was performed using agar disk diffusion. The Congo red agar method was used to determine the ability of the UPEC to form biofilm.

Results: The most prevalent UPEC strains in this study belonged to phylogenetic group B2 (58.7%), followed by group C (12.5%), group E (12.0%), and the other groups (16.8%). Among adhesin genes, the prevalence of *fimH* (91.8%) was highest, followed by *pap* (79.3%), *sfa* (12.0%), and *afa* (7.7%). The rates of resistance to fluoroquinolones, trimethoprim-sulfamethoxazole, and amoxicillin-clavulanate were ~65%, 54.3%, and 36.5%, respectively. The presence of adhesin genes and antibiotic resistance were more frequent in groups B2 and C compared to the other groups. Of the 129 multidrug-resistant UPEC strains, 54% were biofilm producers. Our findings further indicated that biofilm production was significantly correlated with the *pap* adhesin gene ($p \leq 0.05$).

Conclusion: These findings provide molecular epidemiologic data, antibiotic resistance profiles, and the potential for biofilm formation among UPEC strains that can inform further development of the appropriate prevention and control strategies for UTIs in this region.

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Abstract

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Introduction

Urinary tract infections (UTIs) are a common bacterial infection, with 150 million UTI cases observed annually worldwide (Stamm & Norrby, 2001). Uropathogenic *Escherichia coli* (UPEC) is the most common causative agent of both uncomplicated and complicated UTIs, accounting for 75% and 65% of cases, respectively (Flores-Mireles et al., 2015). Clermont and colleagues developed a new polymerase chain reaction (PCR)-based method to classify the eight phylogenetic groups of *E. coli*, of which seven are clustered in *E. coli sensu stricto* (A, B1, B2, C, D, E, and F) and one belongs to *Escherichia* Clade 1 (Clermont et al., 2013). Several studies have reported that phylogenetic groups B2 and D are associated with extraintestinal infection, while the other groups are more prevalent among diarrheagenic and commensal bacteria (Picard et al., 1999; Kumar, Nahid, & Zahra, 2017; Ahumada-Santos et al., 2020).

Adherence and colonization are the crucial steps in UTI pathogenesis. UPEC generally use various adhesins to recognize uroepithelium cells and mediate colonization (Flores-Mireles et al., 2015). Type 1 fimbriae consist of a major protein, FimA that is associated with the ancillary proteins FimF, FimG, and the adhesin FimH, all of which are encoded by the *fim* gene cluster (Orndorff & Falkow, 1984). The P fimbriae are encoded by the *pap* gene cluster, which contains 11 genes (*papA* to *papK*) (Fernández & Berenguer, 2000). P fimbriae promote early colonization of the epithelial cells lining the tubules, while type 1 fimbriae appear to play a role in inter-bacterial binding and biofilm formation (Melican et al., 2011). The S fimbriae are expressed by the *sfa* operon, which was reported to be most often found in *E. coli* strains implicated in human meningitis and septicemia (Antao, Wieler, & Ewers, 2009). The P-independent, X-binding fimbrial adhesin encoded by the *afa1* operon mediates specific binding to uroepithelial cells and human erythrocyte receptors (Labigne-Roussel & Falkow, 1988).

Different studies have investigated the presence of the adhesion-encoding genes *pap* (P fimbriae), *sfa* (S fimbriae), *afa* (afimbrial adhesin), and *fimH* (type 1 fimbriae) across UPEC strains using multiplex PCR (Rahdar et al., 2015; Dadi et al., 2020; Tarchouna et al., 2013; Shetty et al., 2014).

Currently, the empirical treatment of UTIs is an issue of concern due to the increasing rates of antibiotic resistance. The resistance to trimethoprim-sulfamethoxazole (TMP-SMZ), ciprofloxacin, and amoxicillin-clavulanate (AMC) among UPEC isolates is higher in developing countries (ranging from ~50% to 85%) than in developed countries (ranging from 3% to 40%) (Kot, 2019). Routine standard antimicrobial susceptibility testing must be performed in order to reduce the rates of inappropriate empirical antibiotic therapy of UTIs and thereby decrease the occurrence of multidrug-resistant (MDR) UPEC (Adamus-Białek et al., 2018).

Biofilms are microbial communities that adhere to various surfaces, and the cells within a biofilm are encased in self-produced extracellular polymeric matrix (Hall & Mah, 2017). The ability of UPEC to form biofilms is important, as biofilms increase antimicrobial agent tolerance and facilitate evasion of the urinary tract host defense, contributing to the evolution of MDR strains and the recurrence of UTIs (Mittal, Sharma, & Chaudhary, 2015).

A study of virulence genes and antimicrobial susceptibility patterns of UPEC in southern Thailand was previously reported (Themphachanal et al., 2015), but there is no information on the new classification of phylogenetic groups or the biofilm-forming ability of UPEC. Therefore, the aim of the present study was to determine the phylogenetic groups, adhesin gene distribution, antimicrobial resistance profiles, and biofilm formation ability of UPEC isolated from patients with UTIs in central Thailand. We also investigated the possible correlation between adhesin genes and the ability to form biofilm.

Materials and Methods

Ethical approval

E. coli strains were isolated from patients with UTI then identified and collected at the Nopparat Rajathanee Hospital as part of the routine microbiological laboratory. The study protocol was approved by the Ethics Review Board (ERB) of the Research Institute of Rangsit University (DPE.No.RSUERB2018-002). All the bacterial strains were acquired with permission from the Director of Nopparat Rajathanee Hospital**.**

Bacterial strains

The 208 non-repetitive *E. coli* strains isolated from urine specimens of UTI patients between February and May 2018 were used from the current study. *E. coli* strains were isolated from pure cultures and identified in the department of microbiological laboratory in the Nopparat Rajathanee Hospital. The bacteria were confirmed as *E. coli* using standard biochemical tests in our laboratory and only one isolate from each patient was investigated.

Characterization of phylogenetic groups and adhesin genes

Bacterial DNA was extracted using the optimized boiling method (Dashti et al., 2009). The phylogenetic groups of *E. coli* were characterized using multiplex PCR according to the protocol previously published (Clermont et al., 2013). Table S1 shows the primer sequences and the size of amplicons. In addition, four adhesin genes, *pap*, *sfa*, *afa*, and *fimH*, were detected in all isolates using multiplex PCR (Yamamoto et al., 1995; Le Bouguenec, Archambaud, & Labigne, 1992; Struve & Krogfelt, 1999). The details of the primers and sizes of PCR products are listed in Table S2. The PCR reaction volume contained 15 µl of 2X AmpMaster^{TM} HS-Taq (GeneAll®,

Korea), 10 pmol/μl of each primer, 3 μl of DNA template, and DNase-free H_2O to a final volume of 30 μl. Amplification was carried out in the Mastercycler® nexus (Eppendorf, Germany) under the following conditions: initial denaturation at 95°C for 3 min, 45 cycles of 45 s denaturation at 95°C, 45 s of primer annealing at 55°C (to characterize the phylogenetic groups) and 54°C (to amplify the adhesin genes), 60 s of extension at 72°C, and further extension for 5 min at 72°C. PCR products were separated on a 2% agarose gel with a 100-bp DNA ladder (Fermentas, US) and visualized on a UV trans-illuminator.

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed using the agar disk diffusion method according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2018). The antibiotic disks (Oxoid, UK) ampicillin (10 μg), amoxicillin-clavulanate (20/10 μg), piperacillin-tazobactam (100/10 μg), cefoperazone/sulbactam (75/30 μg), cefazolin (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), ceftazidime (30 μg), imipenem (10 μg), meropenem (10 μg), ertapenem (10 μg), gentamicin (10 μg), amikacin (30 μg), netilmicin (30 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), norfloxacin (10 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), and fosfomycin (200 μg) were used. *Escherichia coli* ATCC 25922 was used as a control in all antibiogram tests. Whether a strain was MDR was determined on the basis of acquired non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012).

Detection of biofilm formation

The biofilm production of all *E. coli* strains was determined using the Congo red agar (CRA) method, as previously published (Arciola et al., 2012). The six color tones of colonies were

137 categorized as follows: very black, black, almost black, which were interpreted as weak,
138 moderate, and strong biofilm producers, respectively, and bordeaux, red, and very red, reported
139 as non-biofilm producers.

140 **Statistical analysis**

141 Descriptive statistics were generated by performing Chi-square tests using SPSS version 21
142 (IBM SPSS Inc., Armonk, NY, USA). Results were considered statistically significant if the *p*-
143 value was ≤ 0.05.

144

145 Results

146 Among 1,926 patients with symptoms of UTI, a total of 208 isolates were identified as *E.*
147 *coli*. The demographic characteristics of patients infected with UPEC are shown in Table 1.
148 Among the patients, 154 (74%) were female and 54 (26%) were male. Patients were stratified
149 into five different age groups, and those over 65 years represented 63.9% of all patients. The
150 highest number of UPEC samples was isolated from catheter urine samples (150, 72.1%). The
151 highest proportion of UPEC isolates came from the internal medicine ward (80, 38.5%),
152 followed by the emergency room (45, 21.6%), intensive care unit (34, 16.3%), and outpatients
153 (22, 10.6%).

154 We characterized the phylogenetic groups of *E. coli* from urine specimens by detecting
155 the *arpA* (400 bp), *chuA* (288 bp), *yjaA* (211 bp), and *TspE4.C2* (152 bp) genes using multiplex
156 PCR (Fig. 1A). Primers specific for the *trpA* (489 bp) gene were added to all PCR reactions to
157 provide an internal control. Groups C and E were classified by amplification of the *trpA* (219 bp)
158 and *arpA* (301 bp) genes using specific primers. The majority of the 208 *E. coli* isolates were
159 group B2 (122, 58.7%), followed by group C (26, 12.5%), group E (25, 12%), group A (10,

4.8%), group F (9, 4.3%), group D (6, 2.9%), group B1 (5, 2.4%), unassignable (3, 1.4%), and clade I or clade II (2, 1.0%; Fig. 1B).

Adhesin-encoding genes were successfully amplified by multiplex PCR. The most frequent UPEC adhesin gene was *fimH* (191, 91.8%), followed by *pap* (165, 79.3%), *sfa* (25, 12.0%), and *afa* (16, 7.7%). We also investigated the adhesin gene patterns of the strains (Table 2). Among the isolates, 30 (14.4%), 167 (80.3%), and 11 (5.3%) possessed 1, 2, and 3 adhesin genes, respectively. A high prevalence of combined *fimH* and *pap* genes was found (69.2%). Moreover, the *fimH* and *pap* genes were commonly found in all phylogenetic groups. The adhesin genes *sfa* and *afa* were absent in groups A and D and in clade I/clade II. The *sfa* gene was predominantly seen in groups F (33.3%) and B1 (20.0%), while the *afa* gene was highly prevalent in group E (32.0%; Table 3).

We performed antimicrobial susceptibility tests on *E. coli* strains using different categories of antibiotics. The rates of antibiotic resistance across the phylogenetic groups are shown in Table 4. All isolates showed high rates of resistance to ampicillin (84.1%), ciprofloxacin (65.4%), norfloxacin (65.4%), levofloxacin (64.9%), trimethoprim-sulfamethoxazole (54.3%), cefazolin (44.7%), cefotaxime (43.8%), ceftriaxone (43.8%), ceftazidime (43.8%), amoxicillin-clavulanate (36.5%), and gentamicin (33.7%). The rates of resistance to other antibiotics were between ~1% and 6%. Group C had the highest rates of resistance to all antibiotics except amikacin, netilmicin, and fosfomycin. Three isolates (1.4%) in group C were carbapenems-resistant. Interestingly, most of the 129 isolates (62.0%) that were MDR and belonged to group B2 (59.7%; 77 of 129).

Using the CRA method, the abilities of bacteria to form biofilm were categorized into four groups based on the color tones of colonies. Among the 95 *E. coli* strains that could form

biofilm, 4 (4.2%) showed strong biofilm-forming ability, 38 (40.0%) showed moderate ability, and 53 (55.8%) showed weak ability. The biofilm-producing stains were predominantly clustered in phylogenetic group B2 (Table 5). Biofilm- and non-biofilm-producing UPEC showed different antimicrobial resistance profiles. Among the biofilm producers, the rate of resistance was highest for ampicillin (90%), followed by fluoroquinolones (82%), cephalosporins (50%), and gentamicin (38%). No biofilm producer was resistant to carbapenems. In contrast, the non-biofilm producers were more resistant to TMP-SMZ (58%), followed by piperacillin-tazobactam (7%) and carbapenems (3%). The frequency distribution is presented in Fig. 2. Of the 129 MDR *E. coli* isolates, 54% were biofilm producers.

We also investigated the association between the four adhesin genes and biofilm formation ability. The results demonstrated that biofilm production was significantly correlated with the *pap* adhesin gene ($p \leq 0.05$; Table 6).

Discussion

The higher proportion of UTIs in female (74%) than male (26%) patients in this study was due to the anatomical structure of the female urethra, which is shorter, wider, and closer to the anus than that of males. *E. coli* is common in the gastrointestinal tract flora and can be easily moved from the anus to the urinary tract, leading to UTIs (Dadi et al., 2020). Half of the UTI cases in this study (50%) were observed in female patients over 65 years of age. In postmenopausal women, the low level of estrogen and high intravaginal pH are associated with increased bacterial adherence to the uroepithelium cell, which causes UTIs (Johansson et al., 1996; Beyer et al., 2001). Our study included a large number of catheter urine specimens, which was correlated with the high percentage of infections in the over-65 age group. The low immunity

level in the elderly puts those of advanced age at a high risk of bacterial infection and is responsible for the high prevalence in catheterized cases (Themphachanal et al., 2015).

Phylogenetic groups B2 and D are common strains implicated in UTIs (Ejrnæs et al., 2011). In contrast to the results of studies from Uruguay and Southern Thailand, where high prevalences of phylogenetic group D were found (Themphachanal et al., 2015; Robino et al., 2014), we observed that group B2 was the most prevalent UPEC (58.7%), followed by group C (12.5%). Our results are in accordance with several studies in which the dominant strain was found to be group B2. These studies were conducted in North America (45% prevalence of group B2) (Johnson et al., 2003), Denmark (67%) (Ejrnæs et al., 2011), Poland (35%) (Kot et al., 2016), South Korea (79%) (Lee et al., 2016), and Ethiopia (30%) (Dadi et al., 2020). Using a novel PCR-based method (Clermont et al., 2013), we could classify UPEC into groups C, E, and F and clade I, resulting in a lower percentage of strains in groups A, B1, and D than in earlier studies. This finding indicates that the triplex method of phylo-grouping misidentifies groups C, E, and F and clade I as belonging to group A, B1, B2, or D (Kumar, Nahid, & Zahra, 2017). Three strains in this study (1.4%) could not be assigned to a phylogenetic group due to large-scale recombination events from two different groups or to genome plasticity driven by loss and gain of genes (Clermont et al., 2013). In this study, 1% of UPEC belonged to cryptic clade I/II. This is a much lower percentage than in a study conducted in Mexico (9%) (Kumar, Nahid, & Zahra, 2017). The cryptic clades are primarily associated with environmental *E. coli*; thus, the observed results may be related to a lack of good hygiene practices. The different distributions of phylogenetic groups may depend on the geographic area, health status of the host, use of antibiotics, and/or variations in research design and sample size of the studies (Derakhshandeh et al., 2013).

The most prevalent adhesin gene was *fimH*, followed by *pap*, *sfa*, and *afa*. In agreement with studies conducted in Ethiopia (Dadi et al., 2020) and Iran (Tajbakhsh et al., 2016), phylogenetic group B2 strains showed the highest frequency of the adhesin genes in our study. We found a coexistence of *fimH* and *pap* genes (69.2%), indicating a high presence of virulence genes among UPEC isolated from UTI patients in Thailand. This outcome was different from that of a study in Iran, in which the combination of *pap* and *afa* virulence genes was more common (Rahdar et al., 2015). The ability of UPEC to form biofilm is a crucial virulence property. We found that 45.7% of UPEC were biofilm producers and that most of these classified into phylogenetic group B2. This finding demonstrates that biofilm formation may be associated with phylogenetic group. The association between biofilm-forming ability and some adhesin genes among UPEC was previously reported (Rahdar et al., 2015; Tajbakhsh et al., 2016; Naves et al., 2008). Consistently, the most significant correlation observed in our study was the correlation between the *pap* gene and biofilm production. In contrast, no significant correlation was seen between the *fimH*, *sfa*, or *afa* genes and biofilm production in the strains evaluated in this study. This finding is in agreement with other studies that did not find significant correlations in clinical isolates of pathogenic *E. coli* (Reisner et al., 2006; Hancock, Ferrie` res, & Klemm, 2007). The discrepant results suggest that these genes are not the only determinants of biofilm production in UPEC strains; rather, environmental and genetic factors may also be involved (Reisner et al., 2006).

It is important to perform antimicrobial susceptibility testing to select the appropriate empiric antibiotic therapy for UTIs. Our findings showed that the rate of resistance to ampicillin (84.1%) was higher than rates of resistance to other antibiotics. In general, fluoroquinolones are recommended for oral antimicrobial therapy in uncomplicated pyelonephritis. TMP-SMZ is

252 commonly used in the treatment of uncomplicated cystitis, while AMC was a first-line therapy
253 for complicated UTIs (Bonkat et al., 2019). However, our results revealed that rates of resistance
254 to fluoroquinolones, TMP-SMZ, and AMC were 65%, 54%, and 37%, respectively. This result is
255 consistent with a previous mini-review reporting increases in resistance rates of those drugs
256 among UPEC isolates in developing countries (Kot, 2019). This likely emerged due to the
257 widespread use of fluoroquinolones for uncomplicated UTIs or the inappropriate use of TMP-
258 SMZ for empiric UTI treatment (Bartoletti et al., 2016). In this study, the strains in phylogenetic
259 group C showed the highest rates of antibiotic resistance. In recent decades, the increasing rate of
260 MDR in UPEC has become a public health threat. A high prevalence of MDR UPEC of
261 approximately 62% was observed in the current study, similar to the findings reported in Iran
262 (60.2%) (Tajbakhsh et al., 2016) and Nepal (63.2%) (Ganesh et al., 2019). Consistently, the
263 majority of MDR UPEC belonged to phylogenetic group B2. Thus, it could be suggested that
264 antimicrobial resistance is compatible with strains that contain virulence genes (Lee et al., 2016).
265 The present study found that biofilm producer strains were more resistant to ampicillin,
266 fluoroquinolones, cephalosporins, and gentamicin than non-biofilm producers. These results
267 were in agreement with previous studies indicating that the sessile bacterial cells are much less
268 susceptible to antimicrobial agents than nonattached (planktonic) cells (Costerton et al., 1999). A
269 higher rate of resistance to TMP-SMZ was found among the non-biofilm producers than among
270 the biofilm producers. One explanation for this finding is that these strains may carry the *dhfr* or
271 *dhps* gene mutation on chromosomal DNA, which are common causes of resistance to this drug
272 (Huovinen et al., 1995).
273 In conclusion, the majority of UPEC among patients with UTIs in this geographical area
274 belonged to phylogenetic group B2. UPEC in this group also showed the highest prevalence of

adhesin genes and biofilm formation. The analysis of the antimicrobial resistance of strains tested in this study showed a high level of resistance to cephalosporins, fluoroquinolones, TMP-SMZ, and AMC among strains belonging to groups B2 and C. Therefore, further study of the molecular epidemiology of UPEC and their antibiotic susceptibility patterns will improve our understanding of the organism and lead to a better management of UTIs.

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References

Adamus-Białek W, Baraniak A, Wawszczak M, Głuszek S, Gad B, Wróbel K, Bator P, Majchrzak M, Parniewski P. 2018. The genetic background of antibiotic resistance among clinical uropathogenic *Escherichia coli* strains. *Molecular biology reports* 45(5):1055–1065. DOI: 10.1007/s11033-018-4254-0.

Ahumada-Santos YP, Báez-Flores ME, Díaz-Camacho SP, Uribe-Beltrán MJ, Eslava Campos CA, Parra-Unda JR, Delgado-Vargas F. 2020 Association of phylogenetic distribution and presence of integrons with multidrug resistance in *Escherichia coli* clinical isolates from

296 children with diarrhoea. *Journal of infection and public health* 13(5):767–772. DOI:
297 10.1016/j.jiph.2019.11.019.

298 Antao EM, Wieler LH, Ewers C. 2009. Adhesive threads of extraintestinal pathogenic
299 *Escherichia coli. Gut pathogens* 1(1):22. DOI: 10.1186/1757-4749-1-22

300 Arciola CR, Campoccia D, Gamberini S, Cervellati M, Donati E, Montanaro L. 2002
301 Detection of slime production by means of an optimised Congo red agar plate test based on a
302 colourimetric scale in *Staphylococcus epidermidis* clinical isolates genotyped for *ica* locus.
303 *Biomaterials* 23(21):4233–4239. DOI: 10.1016/s0142-9612(02)00171-0.

304 Bartoletti R, Cai T, Wagenlehner FM, Naber K, Bjerklund Johansen TE. 2016. Treatment
305 of urinary tract infections and antibiotic stewardship. European Urology Supplements 15(4):81–
306 7. DOI: 10.1016/j.eursup.2016.04.003.

307 Beyer I, Mergam A, Benoit F, Theunissen C, Pepersack T. 2001. Management of urinary
308 tract infections in the elderly. *Zeitschrift fur Gerontologie und Geriatrie* 34(2):153–157. DOI:
309 10.1007/s003910170080.

310 Bonkat G, Bartoletti RR, Bruyère F, Cai T, Geerlings SE, Köves B, Schubert S,
311 Wagenlehner F. 2019. EAU Guidelines on Urological infection [Internet]. Arnhem (The
312 Netherlands): European Association of Urology. Available at https://uroweb.org/wp-
313 content/uploads/EAU-Guidelines-on-Urological-infections-2019.pdf. [accessed 2020 June 20].

314 Clermont O, Christenson JK, Denamur E, Gordon DM. 2013. The Clermont *Escherichia*
315 *coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-
316 groups. *Environmental microbiology reports* 5(1):58–65. DOI: 10.1111/1758-2229.12019

317 Clinical and Laboratory Standards Institute (CLSI). 2018. Performance standards for
318 antimicrobial susceptibility testing. 28th ed. CLSI supplement M100. Wayne, PA: Clinical and
319 Laboratory Standards Institute.

320 Costerton JW, Stewart PS, Greenberg EP. 1999. Bacterial biofilms: a common cause of
321 persistent infections. *Science* 284(5418):1318–1322.

322 Dadi BR, Abebe T, Zhang L, Mihret A, Abebe W, Amogne W. 2020. Distribution of
323 virulence genes and phylogenetics of uropathogenic *Escherichia coli* among urinary tract
324 infection patients in Addis Ababa, Ethiopia. *BMC infectious diseases* 20(1):108. DOI:
325 10.1186/s12879-020-4844-z.

326 Dashti AA, Jadaon MM, Abdulsamad AM, Dashti HM. 2009. Heat treatment of bacteria:
327 a simple method of DNA extraction for molecular techniques. *The Journal of the Kuwait*
328 *Medical Association* 41(2):117–122.

329 Derakhshandeh A, Firouzi R, Moatamedifar M, Motamedi A, Bahadori M, Naziri Z.
330 2013. Phylogenetic analysis of *Escherichia coli* strains isolated from human samples. *Molecular*
331 *Biology Research Communications* 2(4):143–149.

332 Ejrnæs K, Stegger M, Reisner A, Ferry S, Monsen T, Holm SE, Lundgren B, Frimodt-
333 Møller N. 2011. Characteristics of *Escherichia coli* causing persistence or relapse of urinary tract
334 infections: phylogenetic groups, virulence factors and biofilm formation. *Virulence* 2(6):528–
335 537. DOI: 10.4161/viru.2.6.18189.

336 Fernández LA, Berenguer J. 2000. Secretion and assembly of regular surface structures in
337 Gram-negative bacteria. *FEMS Microbiology Reviews* 24(1):21-44. DOI:
338 10.1111/j.15746976.2000.tb00531.x

339 Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. 2015. Urinary tract infections:
340 epidemiology, mechanisms of infection and treatment options. *Nature reviews. Microbiology*
341 13(5):269–284. DOI: 10.1038/nrmicro3432

342 Ganesh R, Shrestha D, Bhattachan B, Rai G. 2019. Epidemiology of urinary tract
343 infection and antimicrobial resistance in a pediatric hospital in Nepal. *BMC infectious diseases*
344 19(1):420. DOI: 10.1186/s12879-019-3997-0.

345 Hall CW, Mah TF. 2017. Molecular mechanisms of biofilm-based antibiotic resistance
346 and tolerance in pathogenic bacteria. *FEMS microbiology reviews* 41(3):276–301. DOI:
347 10.1093/femsre/fux010.

348 Hancock V, Ferrie` res L, Klemm P. 2007. Biofilm formation by asymptomatic and
349 virulent urinary tract infectious *Escherichia coli* strains. *FEMS microbiology letters* 267(1):30–
350 37. DOI: 10.1111/j.1574-6968.2006.00507.x.

351 Huovinen P, Sundström L, Swedberg G, Sköld O. 1995. Trimethoprim and sulfonamide
352 resistance. *Antimicrobial agents and chemotherapy* 39(2):279-289. DOI:10.1128/aac.39.2.279

353 Johansson C, Molander U, Milsom I, Ekelund P. 1996. Association between urinary
354 incontinence and urinary tract infections, and fractures in postmenopausal women. *Maturitas*
355 23(3):265–271. DOI: 10.1016/0378-5122(95)00982-5.

356 Johnson JR, Kuskowski MA, Owens K, Gajewski A, Winokur PL. 2003. Phylogenetic
357 origin and virulence genotype in relation to resistance to fluoroquinolones and/or extended-
358 spectrum cephalosporins and cephamycins among *Escherichia coli* isolates from animals and
359 humans. *The Journal of infectious diseases* 188(5):759–768.

Kot B, Wicha J, Gruzewska A, Piechota M, Wolska K, Obrebska M. 2016. Virulence factors, biofilm-forming ability, and antimicrobial resistance of urinary *Escherichia coli* strains isolated from hospitalized patients. *Turkish journal of medical sciences* 46(6):1908–1914.

Kot B. 2019. Antibiotic Resistance among Uropathogenic *Escherichia coli. Polish journal of microbiology* 68(4):403–415. DOI: 10.33073/pjm-2019-048

Kumar N, Nahid F, Zahra R. 2017. Association of virulence factors, phylogenetic groups and antimicrobial resistance markers in *Escherichia coli* from Badin city, Pakistan. *Journal of chemotherapy* 29(1):8-13. DOI: 10.1080/1120009X.2016.1154682

Labigne-Roussel A, Falkow S. 1988. Distribution and degree of heterogeneity of the afimbrial-adhesin-encoding operon (afa) among uropathogenic *Escherichia coli* isolates. *Infection and immunity* 56(3):640–648

Le Bouguenec C, Archambaud M, Labigne A. 1992. Rapid and specific detection of the *pap*, *afa*, and *sfa* adhesin-encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. *Journal of clinical microbiology* 30(5):1189–1193.

Lee JH, Subhadra B, Son YJ, Kim DH, Park HS, Kim JM, Koo SH, Oh MH, Kim HJ, Choi CH. 2016. Phylogenetic group distributions, virulence factors and antimicrobial resistance properties of uropathogenic *Escherichia coli* strains isolated from patients with urinary tract infections in South Korea. *Letters in applied microbiology* 62(1):84–90. DOI: 10.1111/lam.12517.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. 2012. Multidrug-resistant, extensively drug-resistant and

382 pandrug-resistant bacteria: an international expert proposal for interim standard definitions for
383 acquired resistance. *Clinical microbiology and infection* 18(3): 268–281. DOI: 10.1111/j.1469-
384 0691.2011.03570.x.

385 Melican K, Sandoval RM, Kader A, Josefsson L, Tanner GA, Molitoris BA, Richter-
386 Dahlfors A. 2011. Uropathogenic *Escherichia coli* P and Type 1 fimbriae act in synergy in a
387 living host to facilitate renal colonization leading to nephron obstruction. *PLoS pathogens* 7(2):
388 e1001298. DOI: 10.1371/journal.ppat.1001298

389 Mittal S, Sharma M, Chaudhary U. 2015. Biofilm and multidrug resistance in
390 uropathogenic *Escherichia coli*. *Pathogens and global health* 109(1):26–29.DOI:
391 10.1179/2047773215Y.0000000001.

392 Naves P, del Prado G, Huelves L, Gracia M, Ruiz V, Blanco J, Dahbi G, Blanco M,
393 Ponte Mdel C, Soriano F. 2008 Correlation between virulence factors and in vitro biofilm
394 formation by *Escherichia coli* strains. *Microbial pathogenesis* 45(2):86–91. DOI:
395 10.1016/j.micpath.2008.03.003.

396 Orndorff PE, Falkow S. 1984. Organization and expression of genes responsible for type
397 1 piliation in *Escherichia coli*. *Journal of bacteriology* 159(2):736–744.

398 Picard B, Garcia JS, Gouriou S, Duriez P, Brahimi N, Bingen E, Elion J, Denamur E.
399 1999. The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection.
400 *Infection and immunity* 67(2):546–553. DOI: 10.1128/iai.67.2.546-553.1999

401 Rahdar M, Rashki A, Miri HR, Rashki Ghalehnoo M. 2015. Detection of *pap, sfa, afa,*
402 *foc*, and *fim* Adhesin-Encoding Operons in Uropathogenic *Escherichia coli* Isolates Collected

from Patients with Urinary Tract Infection. *Jundishapur journal of microbiology* 8(8): e22647. DOI: 10.5812/jjm.22647.

Reisner A, Krogfelt KA, Klein BM, Zechner EL, Molin S. 2006. In vitro biofilm formation of commensal and pathogenic *Escherichia coli* strains: impact of environmental and genetic factors. *Journal of bacteriology* 188(10):3572–3581. DOI:10.1128/JB.188.10.3572-3581.2006

Robino L, García-Fulgueiras V, Araujo L, Algorta G, Pírez MC, Vignoli R. 2014. Urinary tract infection in Uruguayan children: Aetiology, antimicrobial resistance and uropathogenic *Escherichia coli* virulotyping. *Journal of global antimicrobial resistance* 2(4):293–298. DOI: 10.1016/j.jgar.2014.08.003.

Shetty AV, Kumar SH, Shekar M, Shetty AK, Karunasagar I, Karunasagar I. 2014. Prevalence of adhesive genes among uropathogenic *Escherichia coli* strains isolated from patients with urinary tract infection in Mangalore. *Indian journal of medical microbiology* 32(2):175–178. DOI: 10.4103/0255-0857.129812.

Stamm WE, Norrby SR. 2001. Urinary tract infections: disease panorama and challenges. *The Journal of infectious diseases* 183 Suppl 1, S1–S4. DOI: 10.1086/318850

Struve C, Krogfelt KA. 1999. In vivo detection of *Escherichia coli* type 1 fimbrial expression and phase variation during experimental urinary tract infection. *Microbiology* 145(Pt 10):2683–2690. DOI: 10.1099/00221287-145-10-2683.

Tajbakhsh E, Ahmadi P, Abedpour-Dehkordi E, Arbab-Soleimani N, Khamesipour F. 2016. Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of

424 uropathogenic *E. coli* isolated from clinical samples in Iran. *Antimicrobial resistance and*
425 *infection control* 5:11. DOI: 10.1186/s13756-016-0109-4.

426 Tarchouna M, Ferjani A, Ben-Selma W, Boukadida J. 2013. Distribution of
427 uropathogenic virulence genes in *Escherichia coli* isolated from patients with urinary tract
428 infection. *International journal of infectious diseases* 17(6):e450e453. DOI:
429 10.1016/j.ijid.2013.01.025.

430 Themphachanal M, Kongpheng S, Rattanachuay P, Khianngam S, Singkhamanan K,
431 Sukhumungoon P. 2015. Molecular characterization of virulence and antimicrobial susceptibility
432 profiles of uropathogenic *Escherichia coli* from patients in a tertiary hospital, southern Thailand.
433 *The Southeast Asian journal of tropical medicine and public health* 46(6):1021–1030.

434 Yamamoto S, Terai A, Yuri K, Kurazono H, Takeda Y, Yoshida O. 1995. Detection of
435 urovirulence factors in *Escherichia coli* by multiplex polymerase chain reaction. *FEMS*
436 *immunology and medical microbiology* 12(2):85–90. DOI: 10.1111/j.1574-
437 695X.1995.tb00179.x.

Table 1(on next page)

Demographic characteristics of patients infected with uropathogenic *E. coli* (N = 208)

Table 1 Demographic characteristics of patients infected with uropathogenic *E. coli* (N = 208)

Parameter		No. of isolates (%)	Chi-square	Degree of freedom	*p*-value
Gender					
	Female	154 (74.0)	48.08[a]	1	<0.0001
	Male	54 (26.0)			
Age (years)					
	<14	11 (5.3)	265.02[b]	4	<0.0001
	15 - 24	6 (2.9)			
	25 - 44	15 (7.2)			
	45 - 64	43 (20.7)			
	≥65	133 (63.9)			
Type of samples					
	Midstream urine	58 (27.9)	40.69[a]	1	<0.0001
	catheter urine	150 (72.1)			
Hospital Unit					
	Out-patient	22 (10.6)	188.23[c]	7	<0.0001
	In-patient Internal medicine	80 (38.5)			
	ER	45 (21.6)			
	ICU	34 (16.3)			
	Surgery	12 (5.8)			
	Pediatrics	8 (3.8)			
	Stroke	5 (2.4)			
	Burn	2 (1.0)			
MDR stains					
	MDR	129 (62.0)	12.02[a]	1	0.001
	Non-MDR	79 (38.0)			

[a] 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 104.0.

[b] 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 41.6.

[c] 0 cells (.0%) have expected frequencies less than 5. The minimum expected cell frequency is 26.0.

Table 2(on next page)

Profiles of adhesin genes in uropathogenic *Escherichia coli* strains.

Table 2 Profiles of adhesin genes in uropathogenic *Escherichia coli* strains.

No. of genes	Adhesin genes patterns	No. of isolates	Total (%)
1	*fimH*	19	30 (14.4%)
1	*pap*	4	
1	*sfa*	5	
1	*afa*	2	
2	*fimH, pap*	144	167 (80.3%)
2	*fimH, sfa*	11	
2	*fimH, afa*	6	
2	*pap, sfa*	2	
2	*pap, afa*	4	
3	*fimH, pap, sfa*	7	11 (5.3%)
3	*fimH, pap, afa*	4	

Table 3(on next page)

Distribution of virulence genes in various phylogenetic groups of uropathogenic *Escherichia coli* isolates.

Table 3 Distribution of virulence genes in various phylogenetic groups of uropathogenic *Escherichia coli* isolates.

Phylogenetic group	**Virulence genes**			
	fimH	***pap***	***sfa***	***afa***
B2 (n = 122), %	116 (95.1)	100 (82)	19 (15.6)	6 (4.9)
C (n = 26), %	24 (92.3)	20 (76.9)	1 (3.8)	0
E (n = 25), %	22 (88)	14 (56)	1 (4)	8 (32)
A (n = 10), %	10 (100)	10 (100)	0	0
F (n = 9), %	6 (66.7)	7 (77.8)	3 (33.3)	1 (11.1)
D (n = 6), %	5 (83.3)	5 (83.3)	0	0
B1 (n = 5), %	5 (100)	4 (80)	1 (20)	0
Unassignable (n = 3), %	2 (66.7)	3 (100)	0	1 (33.3)
CladeI (n = 1), %	1 (100)	1 (100)	0	0
Clade I or II (n = 1), %	0	1 (100)	0	0
Total (n = 208), %	**191 (91.8)**	**165 (79.3)**	**25 (12.0)**	**16 (7.7)**

Table 4(on next page)

Prevalence of antimicrobial resistance among various phylogenetic groups of uropathogenic *Escherichia coli* isolates.

1 **Table 4** Prevalence of antimicrobial resistance among various phylogenetic groups of uropathogenic *Escherichia coli* isolates.

Antimicrobial resistance	**Phylogenetic group**									
	B2	C	E	A	F	D	B1	Unassignable	CladeI and I or II	Total
	n = 122, %	n = 26, %	n = 25, %	n = 10, %	n = 9, %	n = 6, %	n = 5, %	n = 3, %	n = 2, %	n = 208, %
Penicillins										
AMP	101 (82.8)	25 (96.2)	21 (84)	8 (80)	6 (66.7)	6 (100)	4 (80)	3 (100)	1 (50)	175 (84.1)
β-lactam/β-lactamase inhibitor combinations										
AMC	39 (32)	17 (65.4)	9 (36)	3 (30)	4 (44.4)	1 (16.7)	3 (60)	0	0	76 (36.5)
TZP	2 (1.6)	7 (26.9)	1 (4)	0	1 (11.1)	0	0	0	0	11 (5.3)
SCF	5 (4.1)	7 (26.9)	1 (4)	0	0	0	0	0	0	13 (6.3)
Cephalosporins										
KZ	54 (44.3)	18 (69.2)	11 (44)	1 (10)	5 (55.6)	2 (33.3)	1 (20)	1 (33.3)	0	93 (44.7)
CTX	53 (43.4)	18 (69.2)	11 (44)	1 (10)	5 (55.6)	1 (16.7)	1 (20)	1 (33.3)	0	91 (43.8)
CRO	53 (43.4)	18 (69.2)	11 (44)	1 (10)	5 (55.6)	1 (16.7)	1 (20)	1 (33.3)	0	91 (43.8)
CAZ	52 (42.6)	18 (69.2)	11 (44)	1 (10)	5 (55.6)	1 (16.7)	2 (40)	1 (33.3)	0	91 (43.8)
Carbapenems										
IPM	0	3 (11.5)	0	0	0	0	0	0	0	3 (1.4)
MEM	0	3 (11.5)	0	0	0	0	0	0	0	3 (1.4)
ERT	0	3 (11.5)	0	0	0	0	0	0	0	3 (1.4)
Aminoglycosides										

CN	43 (33.6)	12 (46.2)	9 (36)	1(10)	4 (44.4)	1 (16.7)	1 (20)	0	0	70 (33.7)
AK	0	0	0	0	1 (11.1)	0	0	0	0	1 (0.5)
NET	0	0	0	0	1 (11.1)	0	0	0	0	1 (0.5)
Fluoroquinolones										
CIP	87 (71.3)	25 (96.2)	10 (40)	4 (40)	5 (55.6)	1 (16.7)	2 (40)	2 (66.7)	0	136 (65.4)
NOR	88 (72.1)	25 (96.2)	10 (40)	4 (40)	5 (55.6)	1 (16.7)	1 (20)	2 (66.7)	0	136 (65.4)
LEV	86 (70.5)	25 (96.2)	10 (40)	4 (40)	5 (55.6)	1 (16.7)	2 (40)	2 (66.7)	0	135 (64.9)
Folate pathway inhibitors										
SXT	61 (50)	20 (76.9)	16 (64)	5 (50)	5 (55.6)	4 (66.7)	0	1 (33.3)	0	113 (54.3)
Fosfomycins										
FOS	0	0	0	1 (10)	0	0	0	0	0	1 (0.5)

Amp, ampicillin; AMC, amoxicillin-clavulanic acid; TZP, piperacillin-tazobactam; SCF, cefoperazone-sulbactam; KZ, cefazolin; CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; CN, gentamicin; CIP, ciprofloxacin; NOR, norfloxacin; LEV, levofloxacin; SXT, trimethoprim-sulfamethoxazole; IPM, Imipenem; MEM, meropenem; ERT, ertapenem; CN, gentamicin; AK, amikacin; NET, netilmicin; FOS, fosfomycin

Table 5(on next page)

Biofilm forming ability among various phylogenetic groups of uropathogenic *Escherichia coli* isolates.

Table 5 Biofilm forming ability among various phylogenetic groups of uropathogenic *Escherichia coli* isolates.

Phylogenetic group	Prevalence of biofilm formation ability			
	Strong (n = 4), %	**Moderate (n = 38), %**	**Weak (n = 53), %**	**Absent (n = 113), %**
B2 (n = 122)	3 (2.5)	36 (29.5)	46 (37.7)	37 (30.3)
C (n = 26)	0	0	3 (11.5)	23 (88.5)
E (n = 25)	0	0	1 (4)	24 (96)
A (n = 10)	0	0	0	10 (100)
F (n = 9)	0	1 (11.1)	1 (11.1)	7 (77.8)
D (n = 6)	0	0	0	6 (100)
B1 (n = 5)	1 (20)	0	1 (20)	3 (60)
Unassignable (n = 3)	0	1 (33.3)	0	2 (66.7)
CladeI (n = 1)	0	0	0	1 (100)
Clade I or II (n = 1)	0	0	1 (100)	0

Table 6(on next page)

Prevalence of virulence genes among various groups of different biofilm formation ability.

Table 6 Prevalence of virulence genes among various groups of different biofilm formation ability.

Virulence genes	Percentage of biofilm formation ability						
	Strong (n = 4), %	**Moderate (n = 38), %**	**Weak (n = 53), %**	**Total (n = 95), %**	**Absent (n = 113), %**	**Pearson Chi-square**	***p*-value**
fimH	4 (100)	37 (97.4)	49 (92.5)	90 (94.7)	101 (89.4)	1.97[a]	0.16
pap	2 (50)	35 (92.1)	44 (83.0)	81 (85.3)	84 (74.3)	3.76[b]	**0.05**
sfa	0	1 (2.6)	6 (11.3)	7 (7.4)	18 (15.9)	3.58[c]	0.06
afa	0	2 (5.3)	3 (5.7)	5 (5.3)	11 (9.7)	1.45[d]	0.23

P-values were calculated using the Pearson Chi-squared test. P-values ≤ 0.05 are indicated in bold.

[a] 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.76.

[b] 0 cells (.0%) have expected count less than 5. The minimum expected count is 19.64.

[c] 0 cells (.0%) have expected count less than 5. The minimum expected count is 11.42.

[d] 0 cells (.0%) have expected count less than 5. The minimum expected count is 7.31.

Figure 1

The distribution of phylogenetic groups among uropathogenic *Escherichia coli* isolates by the new Clermont phylo-typing method.

(A) Multiplex PCR profiles for specific uropathogenic *Escherichia coli* isolates by detecting the *arpA* (400 bp), *chuA* (288 bp), *yjaA* (211 bp), and *TspE4.C2* (152 bp) genes. Lane M, 100-base pair ladder (Fermantas); Lane 1, group B2 (-, +, +, +); Lane 2, group B1 (+, -, -, +); Lane 3, group D or E (+, +, -, -); Lane 4, group B2 (-, +, +, +); Lane 5, group D or E (+, +, -, -); Lane 6, group B2 (-, +, +, +); Lane 7, group B2 (-, +, +, +); Lane 8, group B2 (-, +, +, +); Lane 9, group A or C (+, -, +, -); Lane NC, negative control. The *trpA* (489 bp) internal control gene appeared in all samples except the negative control. **(B)** The pie chart show the percentage of phylogenetic groups among uropathogenic *Escherichia coli* isolates.

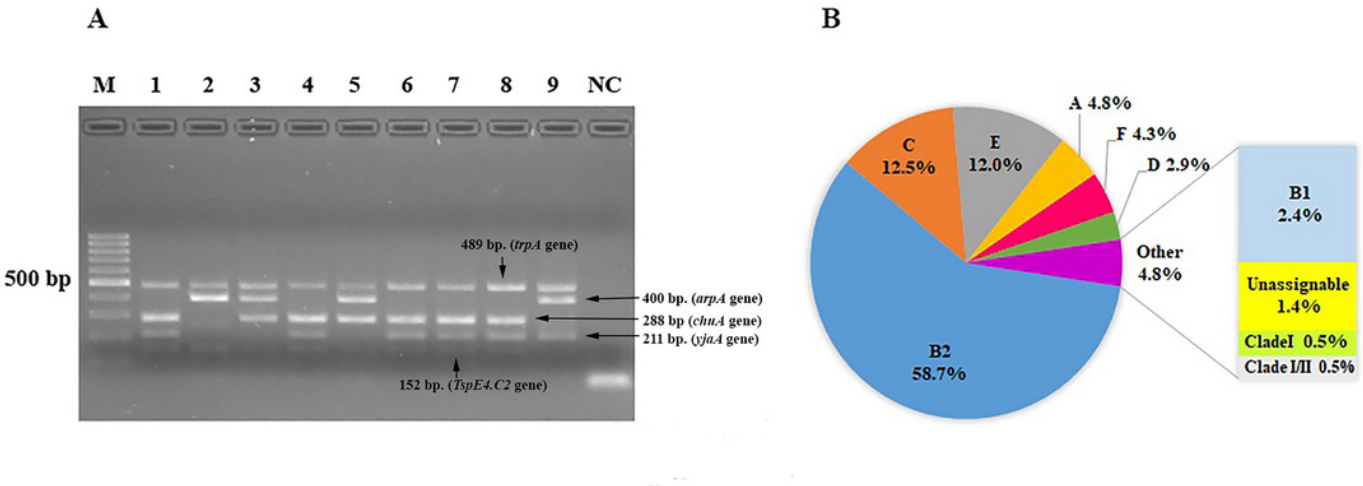

Figure 2

Comparison of antibiotic resistance between biofilm producers and non-biofilm producers.

Bar graphs show the percentage of antibiotic resistance among biofilm producers in blue and non-biofilm producers in orange.