Molecular Characterisation of Uropathogenic Escherichia coli Reveals Emergence of Drug Resistant O15, O22, and O25 Serogroups

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Abstract

Background Uropathogenic Escherichia coli (UPEC) are common pathogens causing urinary tract infections (UTIs). We aimed to investigate the relationship among clinical manifestation, serogroups, phylogenetic groups, and antimicrobial resistance among UPEC. Methods One-hundred Escherichia coli isolates recovered from urine and ureteral scrapings were used for the study. The prevalence of antimicrobial resistance was determined by using EUCAST recommendations. E. coli serogroups associated with UTI, as well as phylogenetic diversity was analysed using multiplex PCR reactions. Results Eighty-seven strains (87%) were isolated from females, while 13 (13%) from males. A high frequency of resistance to cephalosporins (43%) and fluoroquinolones (31%) was observed. Among UTI-associated serogroups, O15 (32.8%), O22 (23.4%), and O25 (15.6%) were dominant and demonstrated elevated resistance rates. The E. coli phylogenetic group B2 was most common. These observations extended to pregnant patients with asymptomatic bacteriuria. Conclusions Due to high rates of resistance, strategies using empirical therapy of second-generation cephalosporins and fluoroquinolones for treatment of UPEC infections should be reconsidered.

Introduction

Extraintestinal *Escherichia coli* (ExPEC) is considered to be the most common aerobic Gram-negative infectious agent responsible for human infections [1]. The ability of ExPEC to cause infections is determined by virulence factors and host susceptibility to infection [2]. ExPEC can exhibit combined pathogenicity pathotypes and utilize different virulence strategies in order to cause various clinical manifestations.

As a common pathotype of ExPEC, uropathogenic *E. coli* (UPEC) mainly originates from intestinal microbiota and can be attributed to the major cause of 75-95% cases of uncomplicated urinary tract infections (UTI), that affect approximately 150 million people worldwide annually [3-4]. High frequency and recurrence of UTI demand extended usage of antibiotics that leads to the development of resistance to the last-line antibiotics [5], as well as the rise of virulent UPEC clones [6-8]. An untreated or mistreated UTI can ascend to kidneys and cause systemic infections with high mortality rates.

Various *E. coli* serogroups have been previously found to be more common between *E. coli* isolated from cases of UTI [9-10]. However little is known on the relationship among antimicrobial resistance and serogroup as well as their relationship to phylogenetic groups and infection types. *E. coli* is phylogenetically classified into four main groups: A, B1, B2 and D [11]. Pathogenic ExPEC strains typically belong to B2 and D groups [12-13], while groups A and B1 are usually associated with commensal strains [14]. B2 and D groups are also considered to be more virulent and susceptible to antibiotics in comparison to those of A and B1 groups [15-16].

Within Baltic region countries little is known about the spectrum of infections caused by UPEC, their molecular epidemiology or patterns of resistance. We therefore aimed to investigate the relationship among clinical manifestation, serogroups, phylogenetic groups, and antimicrobial resistance among UPEC in Lithuania.

Materials And Methods

Escherichia coli Isolates

A collection of total 100 clinically unrelated *E. coli* strains, isolated from the 98 urine samples and 2 ureteral scrapings were used. Isolates were collected during the period of 2017–2018 in from seven medical centres in five different cities of Lithuania. Isolates were recovered from previously anonymized patients undergoing routine medical examination. All received strains were confirmed as *E. coli* by biochemical tests (RapID ONE System, ThermoFisher Scientific) and were stored at -80°C in commercial cryopreservation media (Prolab Diagnostics).

Study Groups

Using the anonymized clinical data provided, all isolates were classified into three groups that were used for further analysis: infectious group (isolates from patients with clinically confirmed UTI and endometritis); non-infectious group (isolates from female patients with asymptomatic bacteriuria; ASB) and unknown diagnosis group (isolates from all sex patients with unavailable medical records). ASB was considered when patients had no clinical symptoms, but two consecutive urine cultures were positive ($\geq 10^5$ CFU/mL) for *E. coli*. The term urinary tract condition (UTC) is used in this study to describe any involvement of a UPEC in the urinary tract.

Evaluation of Antimicrobial Susceptibility

Antimicrobial susceptibility to 11 antibiotics was evaluated by disk diffusion method and interpreted by EUCAST standard [17]. Susceptibility to ampicillin (AMP), amoxicillin/clavulanate (AMC), cefuroxime (CXM), ciprofloxacin (CIP), amikacin (AK), gentamicin (CN), tobramycin (TOB), nitrofurantoin (F), trimethoprim (TMP), imipenem (IPM), and meropenem (MEM) was evaluated. Antibiotic discs were purchased from Liofilchem.

Preparation of Template DNA

Template DNA was prepared by thermal lysis method [18] and used for all PCR reactions. Briefly, 2-3 colonies of *E. coli* were suspended in 300 µL of nuclease-free water. Samples were incubated at 100°C water bath for 10 min and centrifuged at 10,000 × *g* for 10 min at 4°C. Prepared lysates were stored at -20°C.

Serogrouping of *E. coli* Isolates by Using Multiplex PCR

2. *coli* serogroups associated with uropathogenicity (O1, O2, O4, O6, O7, O8, O15, O16, O18, O21, O22, O25, O75, O83) were determined by multiplex PCR, as described by Li *et al*. [19]. Two separate PCR mixes, containing approx. 50–100 ng of template DNA (3 µL), were used for

molecular serogrouping. They were prepared, as described by Li *et al.*, by mixing 1×*Taq* buffer with KCl (ThermoFisher Scientific), 2.5 mM $MgCl_2$, 0.3 µM of each dNTP (ThermoFisher Scientific), 0.05–0.13 µM of the respective primers (Metabion), 2 U of *Taq* DNA polymerase (ThermoFisher Scientific), and adding nuclease-free water until final volume of 30 µL.

Phylogenetic Grouping of *E. coli* Isolates

Phylogenetic grouping was performed by using multiplex PCR, as described by Clermont *et al.* [20]. Reaction was performed in 20 µL of PCR mix containing 200 ng of template DNA. PCR mix was prepared, as described above, by mixing 1×*Taq* buffer with KCl (ThermoFisher Scientific), 1.25 mM of $MgCl_2$, 2 µM of each dNTP (ThermoFisher Scientific), 20 µM of primers (Metabion), 2.5U of *Taq* polymerase (ThermoFisher Scientific), and nuclease-free water.

Statistical Analysis

De-identification of patient data was performed using Caristix HL7 software at each of the primary collaborating centres before submitting the isolates to the laboratory. R statistical software (version 3.5.3) was used for analysing and modelling the data. Binomial testing was used for comparing the proportions of females versus males among the samples pertaining to *E. coli* urinary tract hosts. Chi-square test was used for analysis of differences in proportions for categorical variables. Ages are expressed in boxplots as means, interquartile ranges and ranges. Data considered significant when $p<0.05$.

Results

Prevalence of *E. coli*-caused Conditions

In total, 98 strains were isolated from urine samples and 2 strains were isolated from uterine scrapings. 87 *E. coli* strains (87%) were isolated from females; whereas, 13 strains (13%) were isolated from males (Fig. 1). *E. coli*-caused conditions were more common among females, than males ($p < 0.05$) in all study groups and demonstrated the sex specific predisposition to infection.

Among 100 isolates, 53 isolates (53%) were recovered from infectious study group patients. 32 were recovered from non-infectious study group patients, and 15 were obtained from patients with unavailable clinical records. The most commonly observed infectious study group (53 cases) consisted of 20 confirmed UTI (37.7%), 19 with pyelonephritis (35.8%), 10 with cystitis (18.9%), two of endometritis and two of pyelonephritis with complications of urosepsis (3.8%), respectively (Table 1).

Thirty-two *E. coli* strains (32%) were isolated from pregnant women, who participated in a prenatal surveillance program. All strains caused ASB at the time of culture. One patient later

developed unspecified UTI. Finally, 15 strains were isolated from patients with unavailable medical data (15%).

Age Distribution among Patients with *E. coli*-caused UTC

Among the 87 females, the age of the patients ranged from 2 to 90 years. Among 13 males, the age ranged from 2 to 89 years. The median age of females was 33 years, while observed median age among males was 44 years (Fig. 1).

Antimicrobial Susceptibility Patterns among UPEC

Among all *E. coli* strains, 86% showed resistance to at least one tested antimicrobial, while 22% of strains were resistant to 3 or more antibiotics. A high frequency of resistance to cephalosporins was observed; e.g., as 43% of strains was resistant to cefuroxime (Table 1). Lower levels of resistance to penicillins were observed; 32% of all tested isolates were resistant to ampicillin and 28% of *E. coli* strains showed resistance to amoxicillin/clavulanate. Ciprofloxacin resistance was detected in 31% of all isolates. Lastly, 16% of isolates showed resistance to trimethoprim. Lower resistance rates to aminoglycosides were observed during this study. The highest rates of resistance among tested aminoglycosides were observed to tobramycin (6%), and followed by amikacin (4%) and gentamicin (3%). Among tested isolates, 3% showed resistance to nitrofurantoin. Furthermore, all isolates were susceptible to carbapenems (meropenem and imipenem). Additionally, intermediate resistance to antibiotics was observed. In general, 25% of all tested *E. coli* demonstrated intermediate susceptibility to at least one antimicrobial. 9% showed intermediate susceptibility to tobramycin, 6% - to amikacin, and 5% - to trimethoprim (Table 1).

Serological Diversity among *E. coli* Strains Isolated from Patients with UTC

Among 100 isolates, 64% of *E. coli* isolates belonged to a serogroup associated with uropathogenicity, while the serogroup of remaining 36% tested *E. coli* isolates was not identified, hence belonged to other serogroups (Table 2). Among UTI-associated serogroups, O15 (32.8%), followed by O22 (23.4%), and O25 (15.6%).

Among all isolates belonged to UTI-associated serogroups and recovered from female patients, O15 (95.2%), O22 (86.7%), and O25 (100%) were the most common. Two (13.3%) out of 13 isolates, obtained from male patients, were identified as *E. coli* O22. Furthermore, 7 out of 13 isolates, obtained from male patients, were from serogroups not commonly associated with uropathogenicity (Table 2).

Serogroups O15 and O22 (17%) were the most prevalent among 53 *E. coli* isolates obtained from the infectious study group (Figure 2). Serogroups O8, O15, O18, O22, O25, of isolates from infectious group with clinically proven cystitis, represented a similar distribution of UTI-associated serogroups (1.9%), while the majority of the isolates represented non-UTI associated serogroups (9.4%). In the

infectious group cases of pyelonephritis, serogroups O15 and O22 were the most prevalent and represented 7.6% and 11.3%, respectively, whereas serogroups of 11.3% were not identified (Fig. 2).

In the cases within the non-infectious study group, serogroups O15 (28.1%) and O22 (15.6%) were the most common isolates obtained from patients with ASB. However, serogroups of 28.1% of the isolates were not identifiable by used assay (Fig. 2).

Increased Multidrug Antimicrobial Resistance Observed among O15 Serogroup

Increased antimicrobial resistance levels were observed among *E. coli* UTI-associated serogroups in comparison to those of non-UTI associated serogroups. Increased resistance frequencies were observed among serogroups O15, O22, and O25. Those serogroups had demonstrated extensive resistance rates (resistance to two and more antibiotics) regardless of the diagnosis. 57.1% of O15 isolates indicated resistance to ciprofloxacin, and 71.4% were resistant to ampicillin. 60% of *E. coli* O22 was resistant to ciprofloxacin (Table 2). When compared to other UTI-associated serogroups, O15 showed significantly higher resistance (p=0.016) to ampicillin and trimethoprim (p=0.027) and was resistant to more than one antimicrobial agent.

Phylogenetic Diversity among *E. coli* Isolates

Phylogenetic group B2 was the most prevalent (50%) among analyzed population of *E. coli*, followed by group D that represented 25% of all isolates. Furthermore, 18% of isolates represented B1, while 7% was grouped as A. A similar proportion of distribution among *E. coli* phylogenetic groups was observed when isolates obtained from female patients were analyzed. A similar distribution was observed in the smaller group of males (Table 3).

54. *coli* B2 was dominant (54.7%) and followed by D (24.5%) in infectious group. Group B2 was prevalent between both sexes in the infectious group (Table 3). The different distribution was observed in the non-infectious group, as group A was considerably more prevalent (12.5%) in comparison to those of other groups and conditions. Groups D and B2 were the most common and followed by low frequencies of group A in the group of unavailable medical data.

Phylogenetic group B2 were common among serogroup O22 (60%) and O25 (50%) isolates (Fig. 3).

Patterns of Antimicrobial Susceptibility among *E. coli* Phylogenetic Groups

Group B1 isolates showed highest resistance to ciprofloxacin (44.4%), while group A reached 28.6%, and was followed by B2 and D (28.0% and 28.0%, respectively). Highest resistance rates to amoxicillin/clavulanate were observed among group D (48.0%), while B1 and B2 reached 22.2% and 22%, respectively. Group A demonstrated lowest resistance rates (14.3%). Increased resistance to

ampicillin was observed among the members of group A (42.9%) and D (36.0%), whereas B1 and B2 demonstrated 27.8% and 30.0% resistance. Isolates of A, D, B2, and B1 groups were highly resistant to cefuroxime (57.1%, 52%, 40%, and 33.3%, respectively) (Table 4). There were no significant differences between phylogenetic groups and association between bacteriuric and non-bacteriuric patient populations.

Discussion

This study highlights the phylogenetic composition of E. coli as well as the rates of antimicrobial resistance and associations of resistance, phylogenetic group, and serogroup with clinical conditions. A significantly increased resistance to β-lactams and fluoroquinolones was demonstrated in serogroups O15, O22, and O25. .The overall levels of resistance to those particular antibiotics call for better consideration for usage those classes of antimicrobials for empiric therapy. We also demonstrated the condition specific E. coli serogroup composition in Lithuania. For the first time, the high distribution of multidrug resistant E. coli serogroup O15 in Lithuania was demonstrated. This studied population in Lithuania demonstrated a wide age distribution from 2 to 90 years. The predominant age group among females with a clinically confirmed infectious process (UTI and endometritis) was 20-39 years. The etiology of UTI among young women might be influenced by intercourse related issues, such as frequent sexual activity, new sex partner, and the usage of spermicides [21-22]. However, an increased incidence of UTI was also observed among older females, aged from 60 to 79 supporting the hypothesis that age is an important factor for predisposition to UTI [23]. A lower frequency of UTI and associated conditions among male patients was observed. UTI and associated conditions are more common among older males, ranging from 40 to 59 years, in comparison with females (Fig. 1). Asymptomatic bacteriuria (ASB) is a common condition in various populations [24-26]. Pregnancy, genitourinary abnormalities or indwelling urinary devices increase the risk to develop ASB. In this study, ASB was not observed among other patients, except pregnant women. Increased ASB prevalence during pregnancy can be explained by an increased susceptibility to infections [27]. Thus, during pregnancy ASB can possibly develop to complicated UTI with life threatening complications. ASB in pregnancy is an independent risk factor for preterm delivery [28]. Moreover, ASB might possess a serious threat to pregnant women and even to neonates, as vertical transmission of UPEC might provoke early-onset neonatal infections [29]. However, treatment of ASB is also associated with a higher frequency of antibiotic resistance [30]. Our data indicate that phylogenetic groups, serogroups, and patterns of antimicrobial resistance are similar in the ASB population and infectious group. These patterns include a relatively high level of antimicrobial resistance, particularly among isolates of serogroup O15. Thus, emerging antimicrobial resistance poses challenges even among pregnant patients with ASB. The great attention should be focused on monitoring prevalence of ASB during the pregnancy. Raising antimicrobial resistance is a global problem. In our study, a high distribution of antimicrobial resistance to β-lactams and fluoroquinolones was observed. These resistance patterns among uropathogenic E. coli have important implications or the Baltic States. Resistance to penicillins was frequently observed in the study. Similar results have been demonstrated by studies conducted in Denmark and Estonia, where resistance to ampicillin was higher in strains isolated among uncomplicated and complicated UTI cases as well as paediatric UTI [31-32]. Notably, the highest resistance rate in E. coli was observed for cefuroxime (Table 1). High levels of resistance to cephalosporins in Lithuania impose a great threat to the population. Giedraitienė et al. demonstrated wide distribution plasmid encoded CTX-M class β-lactamases in Lithuanian hospitals [33]. Distribution of the CTX family of β-lactamases in these sectors can possibly explain our observed resistance rates to cefuroxime, since the resistant strains can possibly circulate between both sectors. Considering the clinical importance of cephalosporins in Lithuania and high frequencies of resistance in both, clinical and veterinary sectors, empirical therapy with oral cephalosporins, as a first-choice antibiotic, should be prescribed with a great caution. The resistance to fluoroquinolones was also observed in this study.

Alarmingly, 36% of isolates, obtained from pregnant women with ASB, showed high level resistance to ciprofloxacin indicating high distribution of resistance among strains of possibly commensal origin or colonisation during frequent hospital visits. The levels of resistance to fluoroquinolones are varies in different European countries from 8% to 10% [34-35]. In our study, resistance to fluoroquinolones was higher in comparison to the most of the European countries (5.8%) [36]. However, similar levels of resistance to ciprofloxacin were observed by Stefaniuk et al. in Poland. (34.2%) [37]. Moreover, a certain relationship with the susceptibility to ciprofloxacin and diagnoses was also observed ($p<0.001$) indicating existence of diagnosis specific resistance patterns that might be associated with treatment practice in Lithuania. We demonstrated that among UTI-associated serogroups O15 was most prevalent and followed by E. coli O22 (Fig. 2; Table 2). We have found that serogroups O15 and O22 were remarkably more resistant to ciprofloxacin (57.1% and 60%, respectively) and ampicillin (71.4% and 20.0%, respectively) (Table 2). Frequent occurrence and highly elevated resistance rates of O15 pose a potentially serious public health threat, as some globally distributed clonal variants of O15 are known to be extensively resistant and invasive [38-40]. We demonstrated that 42.8% of highly resistant O15 and 23.8% of O22 isolates were recovered from pregnant females with asymptomatic bacteriuria, suggesting a capability of resistant serovars to successfully colonize the host. Moreover, O15 and O22 were also prevalent among pyelonephritis cases; therefore, under certain conditions O15 and O22 can be responsible for complicated UTI and life-threatening conditions (Fig. 2). Numerous studies have demonstrated that commensal, however, more resistant strains, fall to phylogenetic groups A and B1, whereas pathogenic extraintestinal E. coli isolates belong to group B2 and, in some cases, D. In our study, 25% of all isolates were typed to commensal-originated E. coli, as 7% of tested isolates were identified as group A and 18% as group B1. We found that B2 and D were also associated with ASB, nevertheless in significantly lower number of cases (28.0%), and followed by pyelonephritis (17.3%). However, these groups were more prevalent in unspecified UTI cases (22.7%) compared to A and B1 (12.0%). The study of Giedraitiene et al. analyzed the phylogenetic diversity of ExPEC in Lithuania. ExPEC isolates, obtained from lower respiratory tract, the urinary tract, sterile body sites, wounds, and other body sites, demonstrated the predominance of B2 phylogenetic group (43.3%), followed by A, D, and B1 (28.9%, 27.8%, and 0%, respectively) [33]. Therefore, the diagnosis-specific phylogenetic composition can be seen in different types of infections caused by ExPEC.

Abbreviations

ExPEC Extraintestinal *Escherichia coli*; UPEC Uropathogenic *Escherichia coli*; UTIs urinary tract infections; CFU/mL colony forming units per 1 mL of urine; UTC urinary tract condition.

Declarations

Ethics approval and consent to participate

The study protocols were approved by the Institutional Review Boards of the Lithuanian Health Sciences University (BMC-LMB(M)-310). Consent of participate is not applicable since discarded laboratory material was used with double depersonalization algorithm that ensures no tractability to the patient identity.

Consent for publication

Not applicable

Availability of data and material

All data of the study has been included into the manuscript. Bacterial isolates are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

RP and TJW drafted the manuscript. RP, VP, RP, RP, RS, LK and RG performed laboratory work. RP, FS, and VP, PK analyzed the data. RP and PK designed the study. All authors read and approved the final manuscript

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Tables

Table 1. Distribution of uropathogenic *Escherichia coli* (UPEC) resistance patterns among different urinary tract manifestations.

Study groups	No. of isolates (%)	Distribution among sexes		Frequency of resistance (%)										
		No. among F (%) [a]	No. among M (%) [b]	AMP	AMC	CXM	CIP	AK	CN	TOB	F	TMP	IPM	MEM
Total isolates of *E. coli*	100 (100)	87 (87)	13 (13)	32 (32)	28 (28)	43 (43)	31 (31)	4 (4)	3 (3)	6 (6)	3 (3)	16 (16)	0 (0)	0 (0)
Infectious group (total)	53 (100)	40 (75.5)	13 (24.5)	19 (35.8)	15 (28.3)	26 (49.1)	15 (28.3)	2 (3.8)	1 (1.9)	4 (7.5)	1 (1.9)	6 (11.3)	0 (0)	0 (0)
Cystitis	10 (18.9)	9 (90)	1 (10)	3 (30)	1 (10)	5 (50)	2 (20)	1 (10)	0 (0)	1 (10)	0 (0)	1 (100)	0 (0)	0 (0)
Endometritis	2 (3.8)	2 (100)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)
Pyelonephritis	19 (35.8)	10 (52.6)	9 (47.4)	8 (42.1)	6 (31.6)	9 (47.4)	7 (36.8)	0 (0)	0 (0)	0 (0)	0 (0)	2 (10.5)	0 (0)	0 (0)
UTI with urosepsis	2 (3.8)	2 (100)	0 (0)	1 (50)	1 (50)	1 (50)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
UTI [c]	20 (37.7)	17 (85)	3 (15)	5 (25)	7 (35)	11 (55)	4 (20)	1 (5)	1 (5)	3 (15)	1 (5)	2 (5)	0 (0)	0 (0)
Non-infectious group (total)	32 (32)	32 (100)	0 (0)	8 (25)	8 (25)	11 (34.4)	12 (37.5)	1 (3.1)	2 (6.3)	2 (6.3)	0 (0)	6 (18.8)	0 (0)	0 (0)
ASB [d]	32 (100)	32 (100)	0 (0)	8 (25)	8 (25)	11 (34.4)	12 (37.5)	1 (3.1)	2 (6.3)	2 (6.3)	0 (0)	6 (18.8)	0 (0)	0 (0)
Unavailable clinical records (total)	15 (100)	15 (100)	0 (0)	5 (33.3)	5 (33.3)	6 (40)	6 (40)	1 (6.7)	0 (0)	0 (0)	2 (13.3)	4 (26.7)	0 (0)	0 (0)
No clin. data [e]	15 (100)	15 (100)	0 (0)	5 (33.3)	5 (33.3)	6 (40)	6 (40)	1 (6.7)	0 (0)	0 (0)	2 (13.3)	4 (26.7)	0 (0)	0 (0)

1. The data in this column indicates the distribution of uropathogenic *E. coli* isolates recovered from female (F) patients among different study groups;
2. The data in this column indicates the distribution of uropathogenic *E. coli* isolates recovered from male (M) patients among different study groups;
3. Confirmed symptomatic urinary tract infection (UTI) was considered when urine cultures were positive $<10^5$ CFU/mL for *E. coli*;
4. Confirmed asymptomatic bacteriuria (ASB) was considered when two consecutive urine cultures were positive ($\geq 10^5$ CFU/mL) for *E. coli*;
5. Isolates recovered with patients with known sex and with no detailed clinical data available.

Abbreviations of antibiotics: AMP- ampicillin, AMC-amoxicillin/clavulanate, CXM-cefuroxime, CIP-ciprofloxacin, AK-amikacin, CN-gentamicin, TOB-tobramycin, F-nitrofurantoin, TMP-trimethoprim, IPM-imipenem, MEM-meropenem

Table 2 Antimicrobial resistance patterns observed among different serogroups of uropathogenic *Escherichia coli* (UPEC) isolated from patients with urinary manifestations caused by UPEC.

E. coli serogroups	No. of isolates (%) per group	Distribution of serogroups among sex		Frequency of resistance (%)										
		No. among F (%)[a]	No. among M (%)[b]	AMP	AMC	CXM	CIP	AK	CN	TOB	F	TMP	IPM	MEM
Non-UTI associated [c]	36 (100)	29 (80.6)	7 (19.4)	0 (0)	16 (44.4)	35 (97.2)	1 (2.8)	2 (5.6)	0 (0)	5 (13.9)	0 (0)	3 (8.3)	0 (0)	0 (0)
UTI associated [d]	64 (100)	58 (90.3)	6 (9.4)	32 (50)	12 (18.8)	8 (12.5)	30 (46.9)	2 (3.1)	3 (4.7)	1 (1.6)	3 (4.7)	13 (20.3)	0 (0)	0 (0)
O2	1 (0.6)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
O4	4 (6.2)	3 (75)	1 (25)	1 (25)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)
O8	4 (6.2)	4 (100)	0 (0)	2 (50)	1 (25)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	2 (50)	0 (0)	0 (0)
O15	21 (32.8)	20 (95.2)	1 (4.8)	15 (71.4)	3 (14.3)	2 (9.5)	12 (57.1)	1 (4.8)	0 (0)	1 (4.8)	1 (4.8)	4 (19.0)	0 (0)	0 (0)
O16	3 (4.7)	3 (100)	0 (0)	2 (66.7)	0 (0)	1 (33.3)	2 (66.7)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
O18	4 (6.2)	3 (75)	1 (25)	2 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
O22	15 (23.4)	13 (86.7)	2 (13.3)	3 (20)	2 (13.3)	1 (6.7)	9 (60)	0 (0)	1 (6.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
O25	10 (15.6)	10 (100)	0 (0)	6 (60)	4 (40)	2 (20)	5 (50)	1 (10)	0 (0)	0 (0)	0 (0)	4 (40)	0 (0)	0 (0)
O83	2 (3.1)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	0 (0)	0 (0)	0 (0)	1 (50)	1 (50)	0 (0)	0 (0)

1. The data in this column indicates the distribution of uropathogenic *E. coli* isolates recovered from female (F) patients among different study groups;
2. The data in this column indicates the distribution of uropathogenic *E. coli* isolates recovered from male (M) patients among different study groups;
3. Non-UTI associated serogroups are defined as all other possible *E. coli* O serogroups than the ones that can be detected using Li *et al.,* 2010 suggested multiplex PCR reaction;
4. *E. coli* serogroups O1, O6, O7, O21 and O75 was not detected so therefore not included in the table.

Abbreviations of antibiotics: AMP- ampicillin, AMC-amoxicillin/clavulanate, CXM-cefuroxime, CIP-ciprofloxacin, AK-amikacin, CN-gentamicin, TOB-tobramycin, F-nitrofurantoin, TMP-trimethoprim, IPM-imipenem, MEM-meropenem

Table 3. Distribution of uropathogenic *Escherichia coli* (*E. coli*) phylogenetic groups A, B1, B2 and D among *E. coli* isolates obtained from female and male patients with urinary tract manifestations caused by *E. coli*.

Phylogenetic groups	Number among Females (%)[a]	Number among Males (%)[b]	Total number of isolates per group
Group A	7 (100)	0 (0)	7
Group B1	14 (77.8)	4 (22.2)	18
Group B2	43 (86)	7 (14)	50
Group D	23 (92)	2 (8)	25

1. The data in this column indicate the distribution of uropathogenic *E. coli* isolates recovered from female patients among different study groups;
2. The data in this column indicate the distribution of uropathogenic *E. coli* isolates recovered from male patients among different study groups.

Table 4. Distribution of Phylogenetic *Escherichia coli* (UPEC) resistance patterns among different urinary tract manifestations.

Phylogenetic groups	Antimicrobial resistance profiles										
	AMP	AMC	CXM	CIP	AK	CN	TOB	F	TMP	IPM	MEM
Group A	3 (42.9)	1 (14.3)	4 (57.1)	2 (28.6)	0 (0)	0 (0)	1 (14.3)	0 (0)	2 (28.6)	0 (0)	0 (0)
Group B1	5 (27.8)	4 (22.2)	6 (33.3)	8 (44.4)	1 (5.6)	0 (0)	1 (5.6)	0 (0)	1 (5.6)	0 (0)	0 (0)
Group B2	15 (30)	11 (22)	20 (40)	14 (28)	1 (2)	3 (6)	2 (4)	2 (4)	6 (12)	0 (0)	0 (0)
Group D	9 (36)	12 (48)	13 (52)	7 (28)	2 (8)	0 (0)	2 (8)	1 (4)	7 (28)	0 (0)	0 (0)

Abbreviations of antibiotics: AMP- ampicillin, AMC-amoxicillin/clavulanate, CXM-cefuroxime, CIP-ciprofloxacin, AK-amikacin, CN-gentamicin, TOB-tobramycin, F-nitrofurantoin, TMP-trimethoprim, IPM-imipenem, MEM-meropenem

Figures

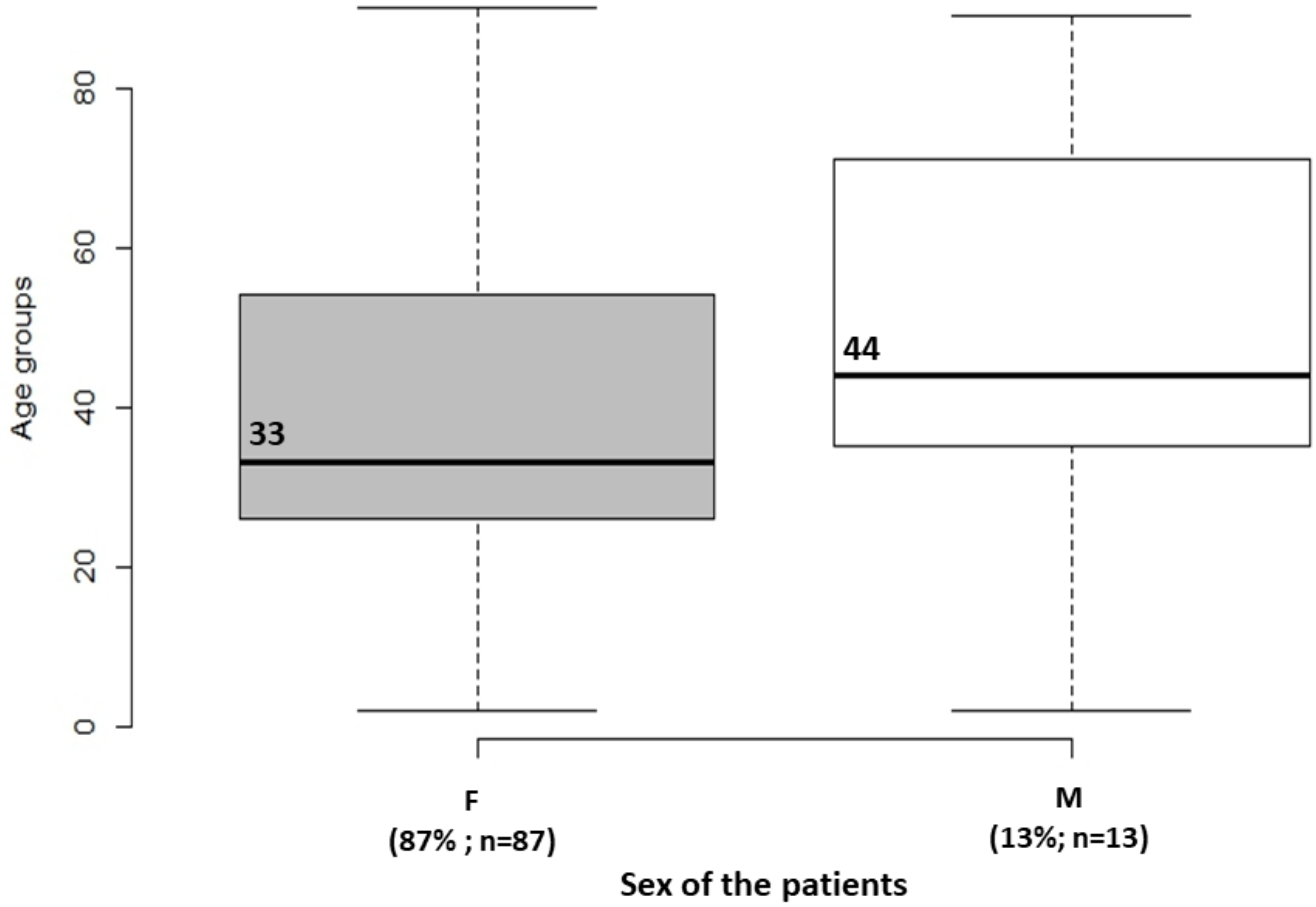

Figure 1

The age distributions 87 female (F) and 13 male (M) patients with E. coli caused conditions. The median age of female patients was 33 years while 44 years was the median age among male patients with E. coli caused conditions. The whiskers in both boxplots indicating lowest and highest age among females (2-90 years) and males (2-89 years).

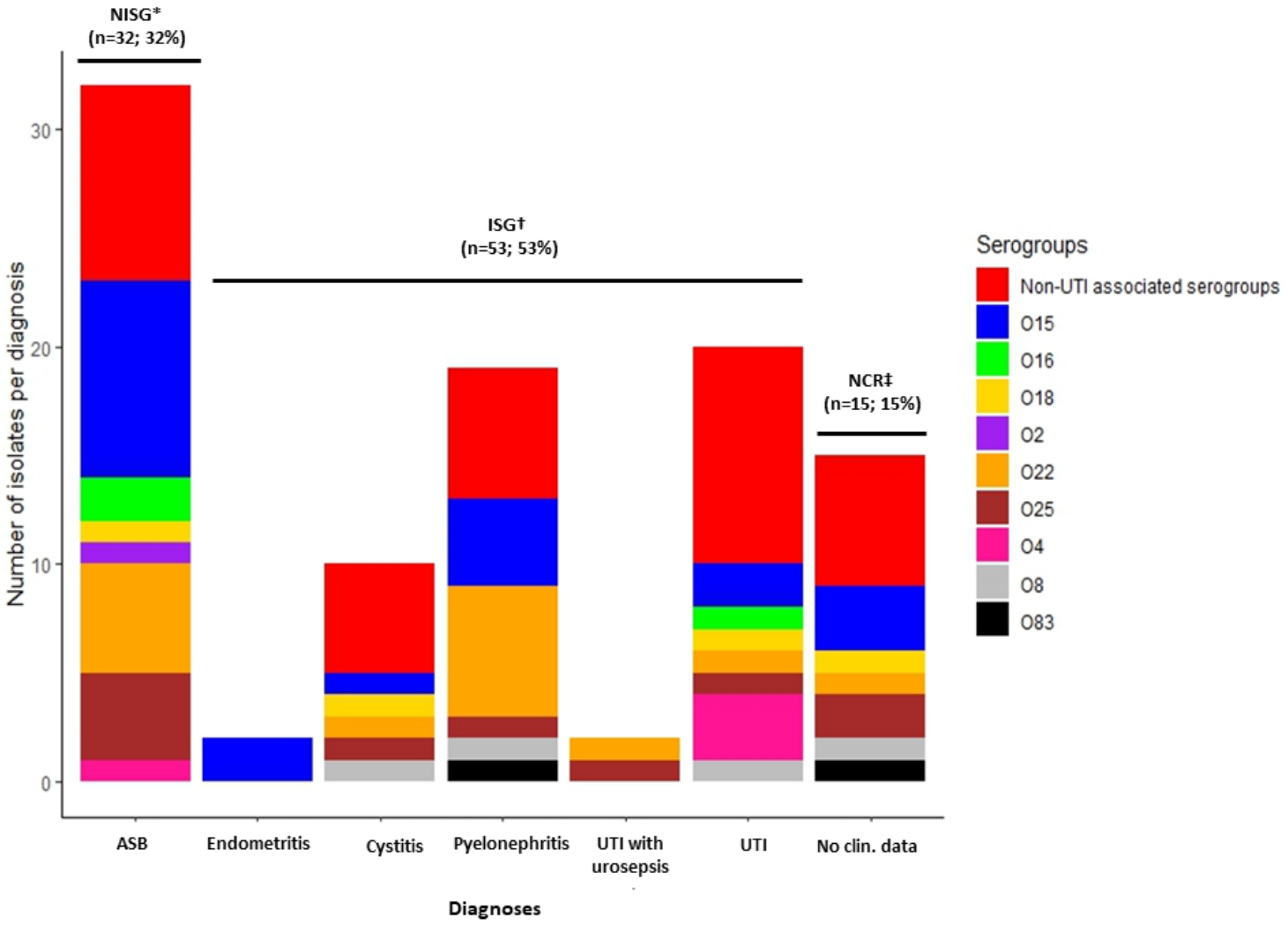

Figure 2

The distribution of E. coli UTI-associated and non-UTI associated serogroups among different clinical conditions. NISG* - non-infectious study group; ISG† - infectious study group; NCR‡ - group with no clinical records available.
Abbreviations of legend: ASB – asymptomatic bacteriuria; UTI – clinically confirmed urinary tract infection.

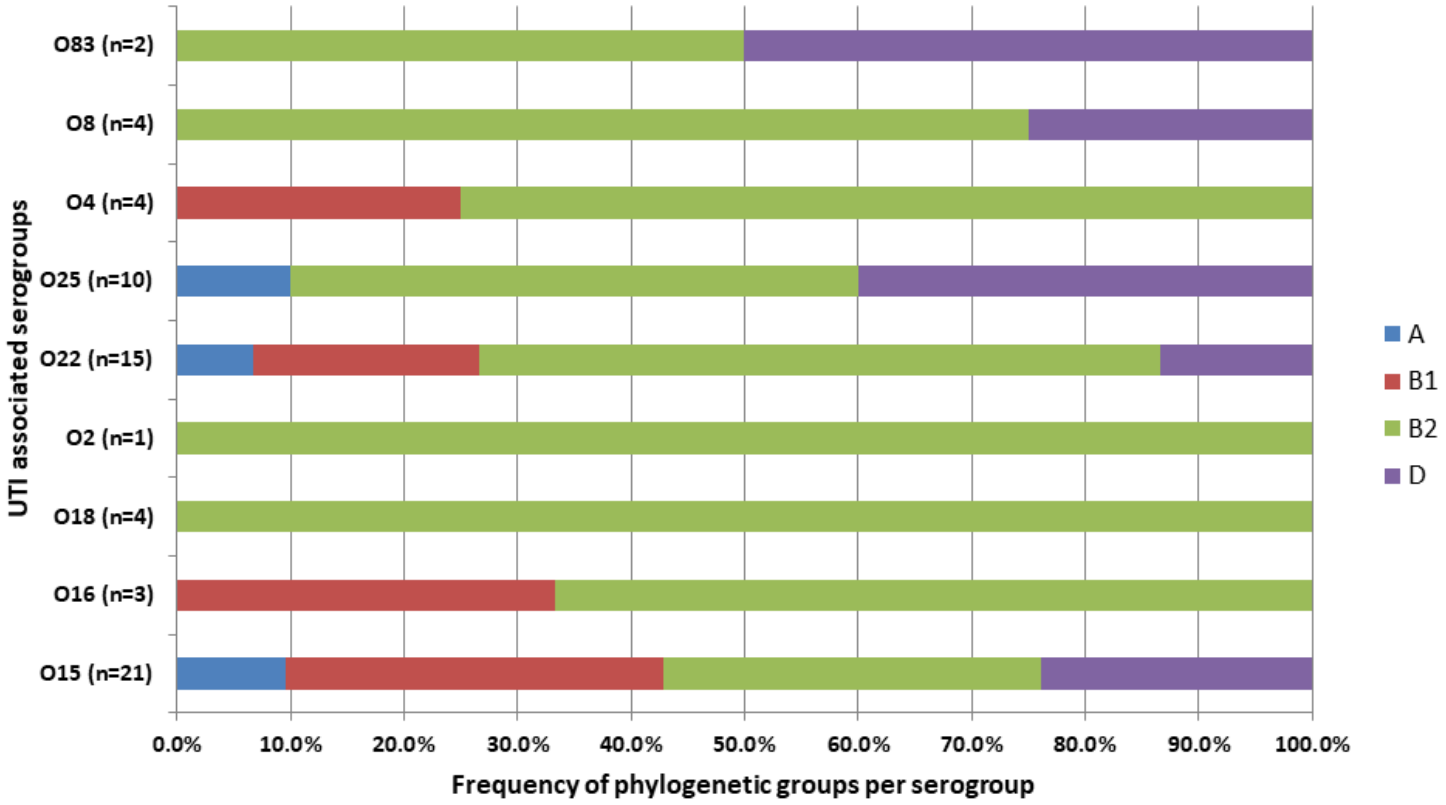

Figure 3

The distribution of phylogenetic groups among E. coli UTI associated serogroups.