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Serotyping and Antibiotic Susceptibility Pattern of Common Bacterial Uropathogens in Urinary Tract Infections in Koohdasht, Lorestan Province

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Abstract

Background: One of the most common diseases worldwide is urinary tract infection (UTI). The main agents causing these infections are bacteria. Urinary tract infections occur when uropathogens colonize the urethra, migrate to the bladder and invade urinary tract cells.

Objectives: The purpose of this study was the detection of uropathogens causing UTIs, as well as serotyping and antibiotic susceptibility of the most common bacteria.

Materials and Methods: The study was performed on 300 urine samples collected from patients referred to Koohdasht Imam Khomeini hospital of Lorestan province. After culturing the samples and determination of uropathogens, antibiotic susceptibility test was performed by the Kirby-Bauer disk diffusion method. Serotyping was performed for the most common uropathogens by polyvalent and monovalent antisera.

Results: Of the 300 samples, 61 samples (20.33%) were positive for UTIs. Among these, 49 samples (80.33%) were Gram-negative bacteria and 12 (19.67%) Gram-positive. The most common uropathogens in UTIs were *Escherichia coli* (55.74%), *Proteus* species (11.47%), *Staphylococcus epidermidis* (11.47%), *Citrobacter* species (8.20%), *Staphylococcus aureus* (8.20%) and *Klebsiella* species (4.92%), respectively. The rate of UTI in females (83.61%) was more than males (16.39%). The highest level of resistance was towards trimethoprim/sulfamethoxazole and the lowest to ampicillin, ciprofloxacin and nitrofurantoin. The most common uropathogen was *Escherichia coli* and the most common serotypes were O142:K86 and O25:K11, respectively.

Conclusions: The treatment of UTIs and resistance control in bacteria should be done based on common strains and choosing an effective antibiotic. Therefore, the determination of prevalent bacterial strains in UTIs of each region based on laboratory tests is very important.

Keywords: Serotyping, Urinary Tract Infection, Uropathogen, Antibiotic Susceptibility

1. Background

Urinary tract infection (UTI) is one of the most common infectious diseases that despite health care, is often associated with recurrence (1). According to the site of infection, UTIs are sub-grouped to bladder infection (cystitis), pyelonephritis and bacteriuria (2). Some UTIs are non-asymptomatic, which can cause symptomatic infections (2).

Bacteria cause nearly 100% of UTIs (2). Studies show that some pathogens such as *E. coli*, *Klebsiella* spp., *Enterococcus* spp., and *Proteus* spp. are the most common bacteria causing these infections (3). *Pseudomonas aeruginosa* is also a pathogen found in UTIs (3). Overall, Enterobacteriaceae are the most common cause of UTI (4).

Uropathogenic *Escherichia coli* (UPEC) is the main agent and causes more than 80% of UTIs (5). *Escherichia coli* serotypes causes of urinary tract infections in a variety of other strains are different O-antigen (6). *Escherichia coli* serotypes causes of UTI are different from other strains for the O-antigen diversity (6). The presence of *E. coli* serotypes is common in pyelonephritis patients (6). Some common *E. coli* O-antigens in UTI include O2, O72, O25, O75, O8, and O16 (6). The O-antigen may be inadequate for the classification of bacterial uropathogens serotypes, hence is also used as capsular antigen (K) and flagellar antigen (H) (6).

There is limited information about the patterns of uropathogens antibiotic resistance (7). Studies show that prescription of antibiotics such as trimethoprim/sulfamethoxazole, cephalosporins and fluoroquinolones is performed regardless to the increase of bacterial resistance (7, 8). The selection of appropriate antibiotics for treatment of UTI is very important (9).

2. Objectives

The aim of this study was to determine the antibiotic susceptibility patterns and serotyping of uropathogens causing UTIs.

3. Materials and Methods

Urine samples were collected from 300 patients with suspected UTI in Koohdasht Imam Khomeini hospital (Lorestan province) during June 2013. Sampling was done using the Midstream Specimen of Urine (MSU) in sterile containers and specimens were maintained at 4°C.

Samples were cultured on a petri of blood agar and MacConkey agar by using a standard calibrated loop (0.01 mL). The urine culture was considered positive when the growth of a type of bacteria was more than 10^5 CFU/mL and more than one type of bacteria 10^4 CFU/mL (10).

Bacteria identification was performed using Gram staining (for bacterial morphology) and differential biochemical tests. For detection of Gram-negative bacteria oxidase, fermentation of sugar, Sulfide-Indole-Motility (SIM), Simmons citrate, Methyl Red/Voges-Proskauer (MR/VP), urease, triple sugar iron (TSI), and lysine decarboxylase tests were used. To identify Gram-positive bacteria catalase, Pyrrolidonyl Arylamidase (PYR), bile esculin agar/6.5% NaCl, novobiocin, SIM, coagulase, and DNase tests were used. Merck and Himedia companies prepared all Differential culture media.

After identifying the bacteria, antibiotic susceptibility test was performed by the Kirby-Bauer disk diffusion based on clinical and laboratory standards institute 2013 (CLSI) (11). This means that after the preparation of a 0.5 McFarland standard solution, samples were cultured, using a sterile swab, on Mueller Hinton Agar medium (Himedia). Finally, antibiotic disks were placed on the medium and plates were incubated at 37°C for 24 hours.

Disks used for Gram-negative bacteria included: nitrofurantoin (300 μg), nalidixic acid (30 μg), ceftriaxone (30 μg), ciprofloxacin (5 μg), ceftazidime (30 μg), gentamicin (10 μg), and trimethoprim/sulfamethoxazole (1.25/23.75 μg). Disks used for Gram-positive bacteria were ciprofloxacin (5 μg), ceftazidime (30 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), chloramphenicol (30 μg), cephalothin (30 μg), ampicillin (10 μg) and tetracycline (30 μg).

All antibiotic disks were prepared by the MAST group. Prevalent uropathogen serotypes were determined by Sifin polyvalent and monovalent antisera (Table 1). For testing, a drop of polyvalent antisera was placed on a sterile slide. Each sample was then added to the antiserum. After 30 seconds, samples were evaluated for agglutination. Complete agglutination was considered as a positive reaction. At the end, the monovalent antisera were used for determination of serotypes.

Table 1. Polyvalent and Monovalent Antisera for *Escherichia coli*

Antisera	
Polyvalent Antisera	
Anti-Coli 1	O26:K60, O44:K74, O114:K90, O125:K70, O142:K86, O158:K-
Anti-Coli 2	O55:K59, O86:K61, O91:K-, O111:K58, O119:K69, O126:K71, O127:K63, O128:K67
Anti-Coli 3	O25:K11, O78:K80, O103:K-, O118:K-, O 124:K72, O145:K-, O157:K-, O164:K-
Monovalent antisera	
ETEC	Anti-Coli O25:K11
EPEC	Anti-Coli O142:K86
EHEC	Anti-Coli O118:K-
EIEC	Anti -Coli O124:K72

Data were analyzed by the SPSS V.19 software using the Chi-square test. Statistical difference was considered at $P \leq 0.05$.

4. Results

Of the 300 cultured urine samples (190 females and 110 males), 61 samples (20.33%) were positive. Of the 61 positive samples, 49 cases (80.33%) were for Gram-negative bacteria and 12 cases (19.67%) for Gram-positive bacteria. The frequency of UTI positive samples was 51 for female cases (83.61%) and 10 for male cases (16.39%). P value for gender difference was significant (0.01) and showed that UTI was related to gender.

Escherichia coli with 34 positive samples (55.74%) and *Proteus* species with seven cases (11.47%) were the most common bacteria causing UTIs (Table 2). *Staphylococcus aureus* with 5 positive cases (8.20%) and *Klebsiella* species with 3 cases (4.92%) did not have an important role in causing UTIs.

The highest resistance was towards trimethoprim/sulfamethoxazole and the lowest to ampicillin, ciprofloxacin and nitrofurantoin. Among Gram-negative uropathogens, the highest and the lowest resistance were related to *Klebsiella* species and *Proteus* species, respectively. Among Gram-positive uropathogens, the highest and the lowest resistance were for *Staphylococcus epidermidis* and *Staphylococcus aureus*, respectively. Antibiotic susceptibility pattern of Gram-positive and Gram-negative bacteria are shown in Tables 3 and 4, respectively.

Table 2. The Frequency of Bacteria in Urinary Tract Infections

Bacteria	No. (%)
Escherichia coli	34 (55.74)
***Proteus* species**	7 (11.47)
Staphylococcus epidermidis	7 (11.47)
***Citrobacter* species**	5 (8.20)
Staphylococcus aureus	5 (8.20)
***Klebsiella* species**	3 (4.92)
Total	61 (100)

Since the most common uropathogen among isolated bacteria was *E. coli*, serotyping was carried for this bacterium. Therefore, of 34 *E. coli* samples, 22 cases showed a positive reaction with anti-coli1 polyvalent antiserum and 12 cases with anti-coli3 polyvalent antisera. The most common serotype was O142:K86 with 16 cases (47.06%) (Table 5).

5. Discussion

Urinary tract infections have led to increased health care costs up to 1.5 billion dollars per annum (12). The main cause of UTI is UPEC (12). Multidrug Resistance (MDR) is expanding in pathogens rapidly (13). Resistance genes transfer in *E. coli* has led to a high resistance in this uropathogen (13). The high prevalence of *bla* CTX-M and *bla* TEM genes in *E. coli* is causing antibiotic resistance in this uropathogen (14).

The diagnosis and treatment of UTIs is very important. Long-term UTIs cause kidney failure (15). Due to lack of proper antibiotic use, treatment should be done based on effective antibiotic selection and the most common bacterial serotypes.

In this study, for 300 samples, the prevalence of bacterial UTI was 61 cases (20.33%), which is a high rate. This study showed that most bacterial UTIs are found in females (83.61%). Antibiotic resistance was high for Gram-negative and Gram-positive uropathogens. The highest antibiotic resistance was to trimethoprim/sulfamethoxazole. Only *Proteus* species were without resistance to ceftazidime, but the resistance of other Gram-negative uropathogens was more than 50% and Gram-negative uropathogens 100%. Among uropathogens, *Klebsiella* species and *S. aureus* have the highest antibiotic resistance. The most common uropathogen and serotype were *E. coli* and O142:K86, respectively.

In Iran and around the world studies have been carried on bacterial UTIs. In one study, the prevalence of bacterial UTI was determined as 8.06% by Khoshbakht et al. from Karaj (2013) (16). In this study similar to our study, most bacterial uropathogen were found in females (88.69%) and the most common uropathogen was *E. coli* (16). In this study similar to other studies, *Klebsiella* was one of the most common causes of UTIs. However in our study, *Klebsiella* species had the lowest frequency among bacteria isolated from UTIs in Koohdasht.

In another study, Aghamahdi et al. reported that the most common uropathogens in UTIs were *E. coli* (59.7%), *Klebsiella* and *Enterobacter* (14.3%), and coagulase-negative *Staphylococcus* (5.2%) in 2013 (17). The most common antibiotic resistance in bacterial uropathogens was against ampicillin and the lowest was ciprofloxacin (17).

In a four-year study, the prevalence of bacterial uropathogens in UTIs was reported as 7.87% by Mirsoleymani et al. in 2014 (18). *Escherichia coli* (65.2%) was the most common uropathogen and the lowest rate of coagulase-positivity was found amongst *staphylococcus* (3.7%) (18). The highest antibiotic resistance in *E. coli* was against cefixime and lowest was against amikacin (18). Motamedifar et al. examined pathogens causing UTIs and antimicrobial susceptibility patterns in Shiraz during 2015 (19). In this study similar to our study, *E. coli* were responsible for more than 50% of UTIs. Antibiotic susceptibility of *E. coli* towards nitrofurantoin 80.9%, gentamycin 77.9% and amikacin 65.3% was reported by Motamedifar et al. (19).

Hryniewicz et al. from Poland also reported *E. coli* as the most common uropathogen (75%) (20). In a study by Lau et al. on 43 uropathogenic *E. coli* strains isolated in the northwest (NW) of England, the most prevalent serogroup was reported O25 with 21 cases (48.84%) (21). In this study unlike our study, among uropathogenic *E. coli* strains has been found O142 serotype. Mandal et al. determined the Prevalence of *E. coli* as 26.01% in UTIs (2012) (22). The highest resistance of *E. coli* was reported towards ampicillin and ciprofloxacin antibiotics (22).

In a 10-year study by Linhares et al. positive bacterial UTIs (12.1%) were reported (3). In this study, antibiotic resistance from 2000 to 2009 had decreased for most antibiotics, yet the prevalence of uropathogens increased (3). Due to the high prevalence of *E. coli* in UTIs, spread of bacterial resistance must be prevented by the use of effective antibiotics. The determination of serotypes causing UTIs and the most effective antibiotic for treatment is essential.

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Table 3. Resistance Rate of Gram-Negative Uropathogens

Name Disk	Cft	Nft	Gm	NA	Cip	Cfz	Tmp/Smx
Escherichia coli	17.65	23.53	61.76	64.70	26.47	50.00	97.06
***Proteus* spp.**	14.29	28.57	71.42	57.14	14.29	0	100
***Citrobacter* spp.**	40.00	40.00	60.00	56.86	40.00	40.00	80.00
***Klebsiella* spp.**	100	66.66	66.66	100	100	66.66	66.66

Abbreviations: Cft, Ceftriaxone; Nft, Nitrofurantoin; Gm,Gentamicin; NA, Nalidixic Acid; Cip, Ciprofloxacin; Cfz, Ceftazidime; TMP/SMX,Trimethoprim/Sulfamethoxazole.

Table 4. Resistance Rate of Gram-Positive Uropathogens

Name Disk	Amp	Clo	Tet	Cfl	Cip	Cfz	Tmp/Smx
Staphylococcus epidermidis	100	57.14	85.71	85.71	42.86	100	85.71
Staphylococcus aureus	100	66.66	100	66.66	66.66	100	100

Abbreviations: Amp, Ampicillin; Cfl, Cephalothin; Cfz, Ceftazidime; Cip, Ciprofloxacin; Clo, Chloramphenicol; TMP/SMX, Trimethoprim/Sulfamethoxazole.

Table 5. The Frequency of Escherichia coli Serotypes in Urinary Tract Infections

Monovalent Antiserum	No. (%)
O142:K86	16 (47.06)
O25:K11	11 (32.35)
Not Detected	7 (20.59)
Total	34 (100)

Footnotes

Authors' Contribution: Study concept, design, acquisition of data, administrative and material support was performed by Siavash Amraei and Seyed Masoud Hashemi Karouei. Critical revision of the manuscript for important intellectual content was carried out by Seyed Masoud Hashemi Karouei. Drafting of the manuscript, statistical analysis, and analysis and interpretation of data was performed by Sajad Babakhani. Mohammad Javad Kazemi was responsible for the study supervision.

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