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Antimicrobial Susceptibility Pattern and Age Dependent Etiology of Urinary Tract Infections in Nemazee Hospital, Shiraz, South-West of Iran

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Background: Urinary tract infections (UTIs) are one of the most common bacterial diseases. The etiological agents and emergence of antimicrobial resistance UTIs vary according to time and geographical region.
Objectives: The present study aimed to determine the prevalence of etiological agents of UTIs and determine their antimicrobial susceptibility pattern to locally available antibiotics.
Patients and Methods: This descriptive cross-sectional study was conducted within six months on all patients with clinical symptoms of UTI who referred to Nemazee Teaching hospital, Shiraz, Iran. Clean catch midstream urine samples were collected and cultured on appropriate media in a short time as possible. Conventional bacteriological methods were followed for the isolation and identification of the bacteria. Antimicrobial susceptibility profiles were determined by locally available antibiotics by using disk diffusion method in accordance with CLSI recommendation.
Results: In this study, from overall 9991 specimens analyzed 848 (8.5%) culture had significant bacterial growth. Of the total positive cultures, 500 (59%) cases were female. *Escherichia coli* (50.6%) was the predominant gram-negative isolated pathogen followed by *Klebsiella* spp. and *Enterobacter* spp., respectively. Enterococcus isolates (11.2%) was the most isolated gram-positive cocci. Nitrofurantoin (80.9%), gentamycin (77.9%) and amikacin (65.3%) had the most in-vitro antibacterial effect on *E. coli* isolates as the predominant cause of UTIs.
Conclusions: The results of regional assessments, such as our study, provide not only useful information for prescription of more effective empirical therapy, but also good epidemiological background for comparison of our situation with other regions.

Keywords: Urinary Tract Infections; Microbial Sensitivity Tests; *Escherichia coli*; Pediatrics; Age

1. Background

Urinary tract infections (UTIs) are one of the most common bacterial diseases nearly recognized to occur in all age groups (1, 2). They may be symptomatic or asymptomatic and the majority of UTIs are not life threatening (3). However, UTIs in vulnerable populations, such as neonates, pregnant women, or the elderly patients, could be associated with significant morbidity and mortality (4-6). Although the UTI is caused by a wide range of pathogens, the most prevalent causative organisms are introduced as gram-negative bacteria that belong to Enterobacteriaceae (1). However, the organisms responsible for UTIs may change over time in terms of epidemiology, etiology, and severity of the condition (1).

UTIs are generally treated empirically by physicians because of the early treatment decreases the further uncomfortable outcome and the rate of morbidity (7). Antibiotic resistance is a growing concern in developing countries especially Iran; as has been noted in previous reports (8-10). Extensive empirical uses of broad-spectrum antibiotics often lead to the outbreaks of resistant isolates to many antimicrobials agents (11). The life-threatening risk of antibiotics resistance among bacteria responsible for UTIs is much critical for specific life time periods or conditions such as elderly age or pregnancy and necessitates an urgent attention (4-6). Despite knowing the risk of antibiotic resistance, since the etiological agents and emergence of antimicrobial resistance in UTIs vary in time and geographical region, periodic monitoring of resistance patterns is a rational way to reduce the risk of failure of antibiotic treatment (2).

2. Objectives

The present study aimed to determine the prevalence of etiological agents of UTIs and determine their antimicrobial susceptibility pattern to locally available antibiotics in Nemazee Teaching Hospital, Shiraz, South-west of Iran.

3. Patients and Methods

3.1. Study Area and Subjects

This cross-sectional study was conducted within six months from August 2012 to February 2013. All patients with clinical symptoms of UTI who referred to Nemazee teaching hospital affiliated to Shiraz University of Medical Sciences were included. Nemazee hospital is a major tertiary care hospital in the South-western Iran with 1000 beds. In total, 9991 urine samples were collected from patients with clinical symptoms of UTI. The patients included in the present study were in the age range of 4 days to 111 years old. We categorized the patients in four groups: neonates and children who were up to two years old, children with age range between 2 - 19 years, patients ranging between 20 - 50 years, and a group containing all the patients older than 51 years.

3.2. Specimens and Bacterial Identification

Clean catch midstream urine samples were collected in sterile disposable tube for adult, sterile urine bags for children and catheter or suprapubic needle aspiration for neonates and analyzed within an hour after transfer to the laboratory. After general examinations using direct microscopy, urine samples were cultured on 5% blood agar and Eosin-Methylene blue (EMB) agar (Merck, Germany) using standard quantitative 10 µL loops and incubated aerobically for 24 hours at 37°C. A urine specimen was defined as culture positive for UTI, when there was a single organism in at least 10^5 colony forming unit (CFU) per milliliter of urine. In the present study, the mixed growth of bacteria was considered as contamination. Conventional bacteriological methods were followed for isolation and identification of the bacteria.

3.3. Antimicrobial Susceptibility Testing

Susceptibility profiles were determined by following locally available antibiotics by using disk diffusion method in accordance with CLSI recommendation (12). Antimicrobial disks (Mast, UK) used for gram positive and negative isolates included ampicillin (10 µg), vancomycin (30 µg), co-trimoxazole (25 µg), gentamicin (10 µg), ciprofloxacin (5 µg), cephalexin (30 µg), nitrofurantoin (300 µg), nalidixic acid (30 µg), norfloxacin (10 µg), cefixime (5 µg), ceftriaxone (30 µg), amikacin (30 µg), and tetracycline (30 µg). The test was performed on Mueller-Hinton agar (Merck, Germany). In our results, intermediate isolates considered as resistant. Frequency of infectious agents and antimicrobial susceptibility were calculated by using SPSS software™ version 19. The results are presented as descriptive statistics in terms of relative frequency.

4. Results

In this study, from overall 9991 specimens which were analyzed, 848 (8.5%) cultures showed a significant bacterial growth. Of the total positive cultures, 500 (59%) cases belonged to female subjects. Gram-negative bacilli were responsible for the majority of UTIs with 647 (76.3%) cases and of which *Escherichia coli* showed the highest isolation rate with 429 cases followed by *Klebsiella* spp. with 93 cases. The gram-positive cocci were the second common causative agent of UTIs and *Enterococcus* spp. accounting for 11.2% (n = 95) of the overall positive cases. The distribution of the isolated pathogen and their relations with patients' age is presented in Table 1.

Table 1. The Distribution of Recovered Isolates According to Patients Age [a]

Groups Isolates	Age ≤ 2	Age 2 - 19	Age 20 - 50	Age ≥ 51	Total	Female
E. coli	65 (50)	82 (54.7)	104 (48.1)	178 (50.6)	429 (50.6)	295 (68.8)
***Enterococcus* spp.**	9 (6.9)	9 (6)	27 (12.5)	50 (14.2)	95 (11.2)	49 (51.6)
***Klebsiella* spp.**	19 (14.6)	14 (9.3)	31 (14.3)	29 (8.3)	93 (11)	55 (59.1)
CoNS	9 (6.9)	15 (10)	19 (8.8)	25 (7.1)	68 (8)	25 (36.8)
***Enterobacter* spp.**	15 (11.5)	8 (5.3)	6 (2.8)	19 (5.4)	48 (5.7)	28 (58.3)
***Acinetobacter* spp.**	1 (0.8)	3 (2)	9 (4.2)	20 (5.7)	33 (3.9)	12 (36.4)
***Pseudomonas* spp.**	6 (4.6)	8 (5.3)	5 (2.3)	11 (3.1)	30 (3.5)	13 (43.3)
***Streptococcus* spp.**	1 (0.8)	4 (2.7)	7 (3.2)	11 (3.1)	23 (2.7)	13 (56.5)
S. aureus	4 (3.1)	3 (2)	4 (1.9)	4 (1.1)	15 (1.8)	3 (20)
***Citrobacter* spp.**	1 (0.8)	1 (0.7)	3 (1.4)	4 (1.1)	9 (1.1)	5 (55.6)
***Proteus* spp.**	0 (0)	3 (2)	1 (0.5)	1 (0.3)	5 (0.5)	2 (40)
Total	130 (100)	150 (100)	216 (100)	352 (100)	848 (100)	500 (59)

[a] Data are presented as No.(%).

E. coli was the predominant isolated pathogen from all age groups. But, the second and third isolated pathogens showed a different pattern in different age groups. *Klebsiella* spp. 14.6% and *Enterobacter* spp. 11.5% were the predominant isolates after *E. coli* among patients aged less than two years old. Coagulase-negative staphylococci (CoNS) with 10% isolation rate had the second place in patients aged 2 - 19 years old and the third place belonged to *Klebsiella* spp. with 9.3%. Among patients in the age range of 20 - 50 years old, *Klebsiella* spp. 14.3% and *Enterococcus* spp. 12.5% were the commonest isolates after *E. coli*. Finally, among patients older than 51 years old, *Enterococcus* spp. and *Klebsiella* spp. had the second and third places of predominant isolated pathogen with the isolation rate of 14.2% and 8.3%, respectively.

Antibacterial susceptibility tests revealed that nitrofurantoin (80.9%), gentamycin (77.9%), and amikacin (65.3%) had the most in-vitro antibacterial effect on *E. coli* isolates as the predominant cause of UTIs. Moreover, gentamycin (77.4%), amikacin (65.6%), and ciprofloxacin (54.8%) were the most effective antibacterial agents against *Klebsiella* isolates. Among gram-negative bacilli, *Acinetobacter* isolates showed the most resistant isolates, since 24 out of 33 screened isolates were resistant to all tested antibiotics. Gentamycin (30.3%) was the effective antibiotic for our tested *Acinetobacter* isolates. The gram-positive cocci displayed a similar susceptibility pattern as they showed a high susceptibility to vancomycin and nitrofurantoin. The results of antibiotic susceptibility patterns for gram positive and negative isolates are presented in Tables 2 and 3, respectively.

Table 2. Antibiotic Susceptibility Pattern of Gram-Positive Isolates Recovered From Cultures [a,b]

Antibiotics	*S. aureus*	*Enterococcus* spp.	*Streptococcus* spp.	CoNS
Vancomycin				
S	15 (100)	28 (29.5)	12 (52.2)	68 (100)
R	0	67 (70.5)	11 (47.8)	0
Nitrofurantoin				
S	13 (86.7)	57 (60)	18 (78.3)	57 (83.8)
R	2 (13.3)	38 (40)	5 (21.7)	11 (16.2)
Ciprofloxacin				
S	4 (26.7)	1 (1.1)	2 (8.7)	31 (45.6)
R	11 (73.3)	94 (98.9)	21 (91.3)	37 (54.4)
Norfloxacin				
S	1 (6.7)	0	0	28 (41.2)
R	14 (93.3)	95 (100)	23 (100)	40 (58.8)
Co-trimoxazole				
S	5 (33.3)	0	0	21 (30.9)
R	10 (66.7)	95(100)	23 (100)	47 (69.1)
Amikacin				
S	6 (40)	2 (2.1)	0	56 (82.4)
R	9 (60)	93 (97.9)	23 (100)	12 (17.6)
Gentamycin				
S	7 (46.7)	17 (17.9)	2 (8.7)	51 (75)
R	8 (53.3)	78 (82.1)	21 (91.3)	17 (25)
Cephalexin				
S	6 (40)	1 (1.1)	2 (8.7)	24 (35.3)
R	9 (60)	94 (98.9)	21 (91.3)	44 (64.7)
Nalidixic acid				
S	1 (6.7)	3 (3.2)	-	1 (1.5)
R	14 (93.3)	92 (96.8)	-	67 (98.5)
Ceftizoxime				
S	6 (40)	3 (3.2)	3 (13)	15 (22.1)
R	9 (60)	92 (96.8)	20 (87)	53 (77.9)
Ceftriaxone				
S	5 (33.3)	5 (5.3)	4 (17.4)	19 (27.9)
R	10 (66.7)	90 (94.7)	19 (82.6)	49 (72.1)
Tetracycline				
S	2 (13.3)	20 (21)	3 (13)	20 (29.4)
R	13 (86.7)	75 (79)	20 (87)	48 (70.6)
Ampicillin				
S	-	21 (21.1)	5 (21.7)	-
R	-	74 (78.1)	18 (78.3)	-

[a] Data are presented as No.(%).
[b] Total numbers of isolates are as follows: *S. aureus*: 15; *Enterococcus* spp.: 95; *Streptococcus* spp.: 23 and CoNS: 68.

Table 3. Antibiotic Susceptibility Pattern of Gram-Negative Isolates Recovered From Cultures [a,b]

Antibiotics	*E. coli*	*Klebsiella* **spp**.	*Enterobacter* **spp**.	*Acinetobacter* **spp.**	*Pseudomonas* **spp.**	*Citrobacter* **spp**.	*Proteus* **spp**.
Ciprofloxacin							
S	183 (42.7)	51 (54.8)	30 (62.5)	6 (18.2)	22 (73.3)	6 (66.7)	3 (60)
R	246 (57.3)	42 (45.2)	18 (37.5)	27 (81.8)	8 (26.7)	3 (33.3)	2 (40)
Gentamycin							
S	334 (77.9)	72 (77.4)	36 (75)	10 (30.3)	24 (80)	6 (66.7)	5 (100)
R	95 (22.1)	21 (22.6)	12 (18.8)	23 (69.7)	6 (20)	3 (33.3)	0
Nalidixic acid							
S	117 (27.3)	37 (39.8)	22 (45.8)	0	0	4 (44.4)	1 (20)
R	312 (72.7)	56 (60.2)	26 (54.2)	33 (100)	30 (100)	5 (55.6)	4 (80)
Norfloxacin							
S	154 (35.9)	45 (48.4)	27 (56.3)	2 (6.1)	21 (70)	5 (55.6)	2 (40)
R	275 (64.1)	48 (51.6)	21 (43.7)	31 (93.9)	9 (30)	4 (44.4)	3 (60)
Cefixime							
S	168 (39.2)	41 (44.1)	15 (31.3)	0	0	4 (44.4)	3 (60)
R	261 (60.8)	52 (55.9)	33 (68.7)	32 (97)	30 (100)	5 (55.6)	2 (40)
Ceftizoxime							
S	196 (45.7)	46 (49.5)	24 (50)	1 (3)	1 (3.3)	6 (66.7)	3 (60)
R	233 (54.3)	47 (50.5)	24 (50)	32 (97)	28 (96.7)	3 (33.3)	2 (40)
Ceftriaxone							
S	182 (42.4)	43 (46.2)	25 (52.1)	0	4 (13.3)	5 (55.6)	4 (80)
R	247 (57.6)	50 (58.8)	23 (47.9)	33 (100)	26 (86.7)	4 (44.4)	1 (20)
Cephalexin							
S	63 (14.7)	26 (28)	8 (16.7)	0	1 (3.3)	3 (33.3)	1 (20)
R	366 (85.3)	67 (72)	40 (83.3)	33 (100)	29 (96.7)	6 (66.7)	4 (80)
Nitrofurantoin							
S	347 (80.9)	18 (19.4)	20 (41.7)	0	0	3 (33.3)	0
R	82 (19.1)	75 (80.6)	28 (58.3)	33 (100)	30 (100)	6 (66.7)	5 (100)
Co-trimoxazole							
S	118 (27.5)	38 (40.9)	24 (50)	0	0	7 (77.8)	1 (20)
R	311 (72.5)	55 (59.1)	24 (50)	33 (100)	30 (100)	2 (22.2)	4 (80)
Tetracycline							
S	43 (10)	12 (12.9)	8 (16.7)	0	2 (6.7)	2 (22.2)	0
R	386 (90)	81 (87.1)	40 (83.3)	33 (100)	28 (93.3)	7 (77.8)	5 (100)
Amikacin							
S	280 (65.3)	61 (65.6)	34 (70.8)	6 (18.2)	19 (63.4)	6 (66.7)	3 (60)
R	149 (34.7)	32 (34.4)	14 (29.2)	27 (81.8)	11 (36.6)	3 (33.3)	2 (40)

[a] Data are presented as No.(%).
[b] Total numbers of isolates are as follows: *E. coli*: 429; *Klebsiella* spp.: 93; *Enterobacter* spp.: 48; *Acinetobacter* spp.: 33; *Pseudomonas* spp.: 30; *Citrobacter* spp.: 9 and *Proteus* spp.: 5.

5. Discussion

The wide range dissemination of new bacterial strains with the ability to resist a wide spectrum of antibiotics would be a serious challenge for physicians encountered with these organisms (13, 14). Implementation of rationalized antimicrobial therapy based on updated antimicrobial susceptibility patterns for routinely used antibiotics could guide the clinicians to better prescribe antibiotics for prophylaxis purposes. In the present study, we tried to provide valuable information on the current causative agents of UTIs and the status of antimicrobial resistance patterns to locally available antibiotics to improving efficient empirical treatment in our region.

In the current study, the bacterial isolation rate from urine samples was found to be 8.5%; that was in accordance with previous isolation rates in Iran (3, 15, 16). However, previous rates of UTIs in other parts of the world were reported in various ranges; this may be a reflection of different infection control policies or other socio-economic factors involved in the studied regions or countries (7, 17, 18). In our results, the positive urine cultures were more frequent among female subjects (59%), as approximately documented in all previous studies (15, 18).

The role of *E. coli* as the predominant causative agent of UTIs was confirmed in most of the previous studies (3, 15-18). In accordance with previous studies, we isolated *E. coli* as the commonest etiologic agent of UTIs in all age groups. However, in our results the patterns for commonest etiologic agent of UTIs other than *E. coli* were different in different age groups; these differences were seen in similar studies.

Farajnia et al. (3) from Tabriz city of Iran introduced *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus saprophyticus* as three common causes of UTIs in patients aged less than 9 years old and for older patients the same pattern was reported with a change in the second place; *Klebsiella* spp. was replaced with *P. aeruginosa* isolates. In our study, *P. aeruginosa* was not found among three major causes of UTIs; however, CoNS (e.g. *S. saprophyticus*) was the second cause of UTIs in patients aged 2 - 19 years old.Mirsoleymani et al. (19) in a hospital survey from Bandar Abbas city, South of Iran reported *E. coli*, *Klebsiella* spp. and *P. aeruginosa* as commonest UTIs among neonates and infants. However, the bacteria isolated from children were as *E. coli*, *Klebsiella* spp. and Staphylococcus coagulase positive. Although the observed pattern among neonates and infants was almost the same as our findings, we isolated *Enterobacter* spp. as the third cause of UTIs among these patients. Only observed difference among children group between our study and Mirsoleymani et al. was about staphylococci, so that CoNS in the current survey was replaced with coagulase positive staphylococci (19).

In accordance with our results Ghorbani et al. (20) from Ahvaz city of Iran, showed *E. coli* and *Enterobacter* spp. as the two common causes of UTIs among infants; however, in their study in contrast with us, they introduced *P. aeruginosa* was among three commonest isolated bacteria in these patients. Moreover, while *E. coli* and *Klebsiella* spp. in their documented patterns among children, teenagers, and adult groups were similar to our findings, but the third causative agent was different from our patterns (20). Among previous Iranian studies on the common isolated pathogens in neonates and pediatric groups, Ghorashi et al. (9) and Kalantar et al. (21) have been shown the most similar results to our study.

In a survey in Turkey the most common identified agents of UTIs among subjects aged 2 months to 18 years old were *E. coli* followed by *Klebsiella* spp., *Enterococcus* spp., and *Enterobacter* spp.; this result was very similar to our work (22). However, in another study from our neighboring country, Pakistan, *Citrobacter* spp. after *E. coli* have been introduced as the most common causative agents of UTIs among infants and children (23).

Previously, similar to our survey Sundvall et al. from Sweden reported *E. coli*, *Klebsiella* spp. and *Enterococcus* spp. as the most frequently isolated bacteria among the elderly patients (24). However, Nowroozi et al. in a survey from elderly residents at nursing home in Tehran city, Iran, reported *E. coli*, *Proteus mirabilis* and *Klebsiella* spp. as the most frequently isolated bacteria from urine cultures (6).

Antibiotic susceptibility results showed *E. coli* isolates as the common cause of UTIs in our study were highly resistant to tetracycline (77.9%), co-trimoxazole (SXT; 77.6%), nalidixic acid (69.9%), and cephalexin (68.1%). On the other hand, *E. coli* isolates were mostly susceptible to nitrofurantoin (80.9%), gentamycin (77.9%), and amikacin (65.3%) which can be considered as highly effective antibiotics for treatment of UTIs in our region. Our antibiotic susceptibility findings are comparable with two separate studies conducted in the north (Karaj city) and south-west (Ahvaz city) of Iran (15, 16). However, our *E. coli* isolates showed a higher resistance to ciprofloxacin and ceftizoxime. Fluoroquinolones (e.g. ciprofloxacin) and SXT are the primary options for effective treatment of UTIs (15, 25). While often resistance towards fluoroquinolones is globally reported with low rates, in-vitro rates of resistance to SXT were noted to be increasingly prevalent in recent decade (25). Such notable resistance (49.4%) to ciprofloxacin against our tested E. coli isolates suggests that this antibiotic must be more cautiously prescribed to prevent increased resistance rates to this effective antibiotic for UTIs treatment. Despite the increasing resistance to ciprofloxacin among our *E. coli* isolates, ciprofloxacin had a good sensitivity for other tested gram-negative bacilli in our study. Also, according to our results, SXT still has a good sensitivity against *Citrobacter* spp. (77.8%), *Enterobacter* spp. (50%), and even *Klebsiella* spp. (40.9%).

In our study, *Acinetobacter* spp. were the most resistant isolates recovered from elderly patients. Therefore, because of more vulnerability of this group of patients, we suggest that antibiotics empirical prescription for UTIs caused by *Acinetobacter* spp. should be postponed until antibiotic susceptibility results be available.

High sensitivity rates to vancomycin and nitrofurantoin among our tested garm-positvie cocci was noted in several previous studies from Iran and other parts of the world (15, 18). Indeed, gentamycin and amikacin showed a good sensitivity against our tested staphylococci.

The findings of the current survey as to increasing resistance rates of uropathogens to antibacterial agents such as ciprofloxacin highlights the variable nature of antibiotic susceptibility patterns according to both in time and geographical area. The results of regional assessments, such as our study, provide not only useful information for prescription of more effective empirical therapy and optimizing institutional infection control policies, but also a good epidemiological background for comparison of our situation with other regions.

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