Original article

Study of bacteria isolated from urinary tract infections and determination of their susceptibility to antibiotics

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Received: June 2009 **Accepted:** September 2009

Abstract

Introduction and objective: Approximately 1 in 3 women will require antimicrobial treatment for a Urinary Tract Infection (UTI) before age 24, and 40% to 50% of women will have a UTI during their lifetime. UTIs in male patients are considered complicated. *Escherichia coli* is the most common cause of UTIs.

Materials and methods: In the present study 7056 patients with clinical symptoms and suspected to UTI were sampled. Clean-Catch midstream urine of the patients was collected. Urine specimens were cultured for isolation of the microbial agents of UTI. The isolated bacteria were identified using biochemical tests. Disk diffusion susceptibility test was used to determine susceptibility of bacterial agents to antibiotics.

Results: In this study 553(8.7%) patients out of 7056 were shown to be urine culture positive (68% females and 32% males)**.** The most isolated bacterium was *E. coli* with frequency rate of 59%. The other bacteria were Klebsiella spp. (11.6%), Enterobacter spp. (9.8%), Pseudomonas spp. (7.2 %), Proteus spp. (2.9%), Acinetobacter spp. (2.7%), Congolese positive Staphylococci (2.2%), Coagolase negative Staphylococci (2.3%), Citrobacter spp. (1.3%) and Streptococci α hemolytic (1.1%). All Gram-negative bacteria were more sensitive to amikacin (90.5-100%). The Gram-positive cocci isolated were more sensitive to tobramycin, kanamycin and ciprofloxacin (100%).

Conclusion: It is concluded that Gram-negative bacilli were responsible for UTI infections in our patients. The most common isolated bacteria from urinary tract infections were *E. coli* and the most effective antimicrobial agents were amikacin, tobramicin and ciprofleoxacin against Gram-negative bacilli and also the most effective antibiotics against Gram-positive cocci were kanamicin, tobramicin and ciprofleoxacin.

Keywords: Bacterial agents, Urinary Tract Infection**,** Antimicrobial susceptibility

Introduction

Urinary tract infection (UTI) is the second most common infectious presentation in community medical practice. Worldwide, about 150 million people are diagnosed with UTI each year, and UTI are classified as uncomplicated or complicated [1]. Uncomplicated UTIs occur in sexually

active healthy female patients with structurally and functionally normal urinary tracts. Complicated UTIs are those that are associated with co morbid conditions that prolong the need for treatment or increase the chances for therapeutic failure. These conditions include abnormalities of the urinary tract that impede urine flow, the existence of a foreign body (e.g., indwelling catheter, stone), or infection with multi-drug resistant pathogens. UTIs in male patients are considered complicated. Despite involvement of the upper urinary tract, pyelonephritis can be considered uncomplicated when it occurs in a healthy patient [2,3].

Urinary tract infection may involve only the lower urinary tract or both the upper and the lower tracts. The term cystitis has been used to describe the syndrome involving dysuria, frequency, and occasionally suprapubic tenderness. Acute pyelonephritis describes the clinical syndrome characterized by flank pain or tenderness, or both, and fever, often associated with dysuria, urgency, and frequency [4]. More than 95% of urinary tract infections are caused by a single bacterial species. *E. coli* is the most frequent infecting organism in acute infection [5,6].

Klebsiella, Staphylococci, Enterobacter, Proteus, Pseudomonas, and Enterococci species are more often isolated from inpatients, whereas there is a greater preponderance of *E. coli* in an outpatient population [7]. *Corynebacterium urealyticum* has been recognized as an important nosocomial pathogen [8]. Anaerobic organisms are rarely pathogens in the urinary tract [9]. Coagulase Negative Staphylococci are a common cause of urinary tract infection in some reports [4]. *Staphylococci saprophyticus* tends to cause infection in young women of a sexually active age [10].

The aims of this study were isolation of pathogenic agents involving UTI and determination of their antibiotics susceptibility pattern in patients referred to Imam Khomeini hospital, Ahvaz, Iran.

Materials and methods

Sampling

In total, 7056 patients with clinical symptoms of UTI referred to Imam Khomeini hospital Ahvaz, Iran, were investigated. There were 4209(50.7%) females and 2847(40.3%) males, with an age range of 25-60 years (mean, 43.7 years). Clean-Catch midstream urine of the patients was collected in a sterile tube (4-5 ml) and immediately transported to the laboratory. Guidelines for proper specimen collection were given to all patients on a printed card [11].

Bacterial colony count of bacteria in UTI

A measured amount of urine, using calibrated loop method was inoculated to nutrient agar medium (Merck, Germany) for colony count. Equal or more than 10^4 CFU/ml of a single potential pathogen or for each of two potential pathogens interpreted as positive UTI and a result of 10^2-10^4 CFU/ml was repeated. A less than 10^2 CFU/ml was interpreted as negative UTI [10]. Urine specimens were cultured for isolation of the microbial agents of UTI on blood agar and MacConky agar media (Himedia, India & Merck, Germany). All the bacteria isolated from urine in this study were identified using conventional biochemical tests [4,11,12].

Antimicrobial susceptibility testing

In the present study antimicrobial susceptibility testing was done on Mueller-Hinton agar (Merck, Germany) using disk diffusion (Kirby Bauer's) technique. This method was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines to determine susceptibility of

UTIs agents [13]. The antibiotic disks (Padtan Teb, Iran and Tehran, Iran) comprised, ampicilin (10µg), kanamicin (30µg), cephalothin (30µg). ciprofleoxacin (5µg), tetracycline (30µg), nalidixic acid (30µg), nitofurantoin (300µg), amikacin (30µg), tobramicin (10µg), ceftriaxone (30µg), cefotaxime (10µg) and gentamicin (10µg) and trimethoprim-sulfamethoxazole (25µg) [11].

Results

In this study, 553(8.7%) patients out of 7056 were showed to be urine culture positive (their colony count was equal or more than 10^4). There were 376(68%) females and 177(32%) males in patients with urine positive culture. Gram-negative bacilli isolated accounted for 522(94.4%) of the positive cultures, while Gram-positive cocci were 5.6%. The frequency of isolated microorganisms and their relation to sex is given in table 1. The most common isolated uropathogens in Gram-negative bacilli and Gram-positive cocci were *E. coli* (59%) and coagulase negative Staphylococci (2.3%) respectively. In this study, the incidence of UTI was ranged in patients between 25-60 (mean, 43.7) years old. The isolated bacterial showed wide differences in their susceptibility to the tested antimicrobial antibiotics. The relation between antimicrobial sensitivity patterns of the isolated bacteria in urine of the population studied is presented in table 2.

Table 1: Frequency of bacterial agents isolated from urine specimens and their relation to sex in this study

Isolated bacteria	No (%)	Female (%)	Male (%)
Gram-negative bacilli			
Escherichia coli	326(59)	75.5	24.5
Klebsiella	64(11.6)	67.7	32.3
Enterobacter	54 (9.8)	65.5	34.5
Proteus	16(2.9)	50	50
Citrobacter	7(1.3)	24.8	57.2
Pseudomonas	40(7.2)	30	70
Acinetobacter	15(2.7)	41.2	58.8
Gram-positive cocci			
Coagulase positive Staphylococci	12(2.2)	69.2	30.3
Coagulase negative Staphylococci	13(2.3)	76.9	23.1
α hemolytic Streptococci	6(1.1)	66.7	33.3
Total	553(100)	68%	32%

Based on results obtained from susceptibility testing (Table 2), all the bacteria recovered from UTI showed the highest degree of resistance to ampicillin, cefalothin. The Gram-negative bacilli isolated from UTI were sensitive to amikacin and, ciprofleoxacin (83%-100%), and Gram-positive cocci were sensitive to kanamicin and tobramicin (100%). The amikacin was more effective against Pseudomonas spp. and Acinetobacter spp. isolated in present study. *E. coli* showed that this bacterium was sensitive to gentamicin, ceftriaxone, amikacin, (90.5%) and to cefotaxime, ciprofloxacin and tobramicin (85.3-89.6%) respectively. Most of the isolated bacteria in this study were resistant to ampicillin (92.4%), cephalothin (80.8%), and tetracycline (73.8%).

Table 2: Antimicrobial sensitivity pattern of bacterial agents isolated from urine specimens

Isolates	AM	KA	CF	CIP	TE	NA	FM	AN	SXT	TOB	CRO	CTX	GM
E. coli	4.9	76.1	25.2	85.3	25.5	77.9	71.2	90.5	53.4	89.6	90.5	89.6	90.5
Klebsiella	18.8	62.5	14.1	85.9	32.5	65.6	23.4	90.6	59.4	75	75	75	65.6
Enterobacter	7.4	57.4	18.5	83.3	25.9	62.9	27.8	94.4	66.7	75.9	69.5	69.5	81.5
Proteus	46.7	73.3	20	100	0.0	73.3	46.7	100	46.8	100	73.3	73.3	100
Citrobacter	0.0	46.7	28.6	85.7	28.6	71.4	46.7	100	46.7	71.4	71.4	57.1	71.4
Pseudomonas	10	22.5	10	60	10	15	15	77.5	7.5	55	27.7	27.5	45
Acinetobacter	0.0	18.8	0.0	22.5	0.0	18.8	25	50	18.8	25	18.8	18.8	18.8
Coa-pos-Staph	0.0	100	50	75	50	50	75	0.0	50	100	75	25	50
Coa-neg-Staph	0.0	100	61.5	100	23.1	0.0	100	69.2	69.2	100	69.2	23.1	100
α-hem-Strep	16.7	100	0.0	33.3	16.7	16.7	16.7	0.0	16.7	100	67.7	0.0	67.7

AM, Ampicilin; KA, Kanamicin; CF, Cephalothin; CIP, Ciprofleoxacin, TE, Tetracycline; NA, Nalidixic acid; FM, Nitofurantoin; AN, Amikacin; SXT, Trimethoprim-sulfamethoxazole; TOB, Tobramicin; CRO, Ceftriaxone; CTX, Cefotaxime; GM, Gentamicin; Coa-pos-Staph, Coagulase positive Staphylococci; Coa-neg-Staph, Coagulase negative Staphylococci; α-hem-Strep, α-hemolytic-Streptococci

Discussion

Urinary tract infections are common conditions worldwide and the pattern of antimicrobial resistance varies in different regions. We describe the relationships between sex, isolated bacterial agents and antibiotic resistance of UTIs. The study was confined to UTIs in adults. In the United States, UTIs account for seven million office visits and 100,000 hospitalizations yearly, making them the most common bacterial infections in outpatient settings [14,15].

Approximately 1 in 3 women will require antimicrobial treatment for a UTI before age 24, and 40% to 50% of women will have a UTI during their lifetime [15]. The estimated annual cost of UTIs is $1.6 billion for evaluation and treatment. Despite advances in antimicrobial therapy, UTIs remain a significant cause of morbidity [14,16]. The sex distribution of patients in our study is consistent with those of other reported studies, showing a statistically predominance of females with UTI (68% of the positive cultures). This result is similar to those reported from many other centers [17]. The elevated incidence of infection among females is related to differences between the male and female genitourinary systems in anatomy and microflora [18].

The uropathogens identified in our study are similar to those of many other studies conducted in different countries either in the region or internationally [19], however different results have been reported. The similarities and differences in the type and distribution of uropathogens may result from different environmental conditions and host factors, and practices such as healthcare and education programmers, socioeconomic standards and hygiene practices in each country.

The prevalence of Gram-positive cocci was not high in our study; this is similar to other studies in different countries [19, 20]. Apart from the Gram-positive isolated in urine, the other isolates are inhabitants of the large bowel. The Enterobacteriaceae family were the most common microorganism isolated of Urinary tract infection in present study accounting 94.4% of total isolated bacteria and amongst them *E. coli* was the most predominant bacteria. There are earlier studies in agreement to present finding [21-25]. In present study a high level of bacterial resistance was seen to ampicillin and cephalothin, which

ampicillin was less effective than cephalothin against isolated bacteria. This is similar to previous studies in the United States [23].

The high prevalence of resistance to the commonly used antibiotics such as ampicilllin, cephalothin and tetracycline has caused considerable alarm [26-28]. The most effective antimicrobial agents in our study and other reported researches were amikacin and ciprofleoxacin [28] for Gram-negative bacilli (81%-100%). However, isolated Gram-positive cocci were fully sensitive to kanamicin and tobramicin. This study is comparable with the results reported by Astal *et al.* [19] and McIsaac *et al.* [29]. Based on the results of this study, it was revealed that the susceptibility of bacteria to ciprofleoxacin and other antibiotics was similar to many studies [19,28-30]. According to our results, the efficacy of amikacin was comparable to other reports [20].

Conclusion

It is concluded that Gram-negative bacilli (Enterobacteracea) were responsible for urinary tract infections and most of the strains were multi-drugs resistant. The most common isolated bacteria from urinary tract infections was *E. coli* and the most effective antimicrobial agents were amikacin, tobramicin and ciprofleoxacin against Gram-negative bacilli and also the most effective antibiotics against Gram-positive cocci were kanamicin, tobramicin and ciprofleoxacin.

Acknowledgment

The authors would like to thank Mrs Mahvash Haidari and personnel of medical microbiology laboratory of Imam Khomeini Hospital for them assistance in this project.

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