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Original Article

Microbial Etiology and Antimicrobial Susceptibility of Bactria Implicated in Urinary Tract Infection in Tehran, Iran

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ABSTRACT

Background and Objectives: **Urinary tract infections (UTI) are one of the most common infectious diseases with different microbial agent and antimicrobial resistant pattern in hospitalized patients and outpatients. In order to assess the adequacy of therapy, knowledge of prevalence and resistance pattern of the bacteria is necessary. The main aim of this study was to evaluate the prevalence and the antimicrobial resistance pattern of main bacterial responsible for UTI in order to establish an appropriate empirical therapy.**

Methods: **All urine samples were referred to Imam Hospital Laboratory, Tehran, Iran during 2011-2012, urine culture isolated and bacteria were identified and the profile of antibiotic susceptibility was characterized.**

Result: **From 1851 urine cultures, UTI was more frequent in woman (68%) E. coli was as usual the most common pathogen implicated in UTI. Most susceptibility was to imipenem (98.9%). nitroforantoin (96%) and amikacin (94.1%) and increased resistance to penicillin (66.6%), nalidixic acid (62.1%) ampicilin (60.1%) and cotrimoxazole 54.3%.**

Discussion: **The most common isolated pathogen was E. coli. According to antibiogram susceptibility, the recommended antimicrobial drugs are nitroforantoin and imipenem. nalidixic acid and cotrimoxazole are not recommended because drug resistance is high.**

Keywords: **Antibiogram, Urinary Tract Infection, Iran**

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Introduction

Urinary tract infection (UTI) is one of the most common infectious diseases of community and also of the hospital setting resulting in high rates of morbidity. Fifty percent of women experience urinary tract infection at least once in their lifetime (1). Some risk factors for UTI are female sex, elevated age, pregnancy and diabetes (1-3).

The most common infection in hospital is UTI due to catherization. Besides, antimicrobial misuse in clinical medicine has increased the microbial resistance and consequently spread bacterial resistance strains. That is a serious public health problem (3-5).

The main cause of UTI is uropathogens such as *E. coli* (46.4%-74.2%), *Klebsiella* SPP. (6-13.45%) *Proteous* SPP. (4.7-11.9%) and *Entrococcous* SPP. (5.3-9.54%). *E. coli* has been identified as the most frequent pathogen in the uncomplicated patient. But *Proteous, Klebsiella, Entrobacter, Seratia* and *Pseudomonas* isolated in recumbent, complicated and catheterized patient. The early treatment of UTI decreased the rate of morbidity. (1, 2, 6-9). In order to prescribe the appropriate antibiotics and prevent antibiotic resistance in patients, it would be necessary to know the model of frequency of microorganisms causing urine infections as well as the model of antibiotic sensitivity and resistance of the microorganisms. Unfortunately there is little publication about the main uropathogen in community acquired UTI and antimicrobial resistance pattern when compared with UTI at hospital level.

Given the growing drug resistance in microorganisms, once-effective antibiotics are less effective on the bacteria causing urinary tract infection. It results from the emergence and expansion of bacteria-resistant strains due to the genetic properties of bacteria, population growth, travel and non-standard administration of antibiotics. The report of sensitivity to anti-microbial factors is submitted to the doctor most often 48 hours after the sample is delivered to the lab. Therefore, in most cases, the treatment is done based on experience and since experimental antibiotic treatment of urinary tract infections must be based on epidemiology and the resistance pattern of uropathlogen, this study is necessary to be conducted.

This information is very important and implies a periodic monitorization in order to decrease the number of therapeutic failure (6-8). The main aim of this study was to evaluate the prevalence and the antimicrobial resistance pattern of the main bacteria responsible for UTI in order to establish an appropriate empirical therapy.

Materials and Methods

We evaluate urine cultures of inpatients & outpatients referred to clinical laboratory of Imam Hospital, Tehran, Iran during the period of 2012-2013.For each patient, the collection date, age, sex, related ward, culture result, identification of bacterial strain and antimicrobial susceptibility test was done. The method of sampling for culture and antibiogram in patients with the urinary control was taking mid-stream sample and from children without sphincter controlling the suprapubic aspiration were done. All samples were studied by a veteran expert in the lab and with the application of standard methods.

The urine sample was inoculated in different culture media. A calibrate loop was dripped in vertical position in the urine sample and the loop was used to inoculate the plate. After incubation, the urine culture when monomorplic bacteria growth was higher than 10^5 cfu/ml, the culture was classified as positive. Additional biochemical tests were performed on the morphology of the isolated bacterial on the results of the microscopic examinations of the gram stained smear. A bacterial suspension in physiology saline solution with turbidity at 0/5 on McFarland scale was prepared. The suspension was spread with a swab on Muller-Hinton agar and antimicrobial disk were placed onto to medium according to isolated organism. The plates were incubated at 37 ^{o}C for 18- 24 h, after incubation the antimicrobial

efficacy was determined by measuring of diameter of the zones of inhibition.

Isolation and Identification of Bacteria

Isolation of these bacteria was performed using Streak-Plate Method on Blood agar and MacConkey agar culture with standard loop (internal diameter 34/1 mm). Culture plates were incubated at 37°C for 24 h. The cultures consisting of more than 10^5 colonies of particular bacteria were considered as positive cultures. The incubation of negative 24 h cultures was extended for another 24 h. Bacteria were identified through performing biochemical tests (indole, citrate, oxidase, and production of H_2S, lysine decarboxylase, fermentation of lactose, urea hydrolysis, gas production, catalase, coagulase, mannitol fermentation and susceptibility testing novobiocin).

Antibiotic Susceptibility Testing

Strains drug resistance evaluation was carried out using disk diffusion method on Mueller-Hinton medium (Merck, Germany). After inoculating the bacteria on Muller-Hinton agar and placing the antibiotic disks, plates were incubated for 24 h in incubator. Then, according to the size of the growth inhibition zone around the disks and international numbers of (NCCLS), results were categorized and reported in three groups :susceptible and Sensitive (S), intermediate susceptibility or sensitivity (I) and resistance (R). *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 were used as control strains and the test results were only accepted when the inhibition zone diameters of the above mentioned control strains were within performance ranges (as described by CLSI No:M100-S16). In case of mixed bacteria, only the major and predominant pathogens were tested. The antibiotics used for susceptibility testing were norfloxacin 10 µg, ofloxacin 5 µg, ciprofloxacin 5 µg, nitrofurantoin 300 µg, co-trimoxazole (SXT), carbenicillin 100 µg, ampicillin 10 µg, cephalothin30 µg, gentamicin 10 µg, amikacin 30 µg, nalidixic acid 30 µg, cefotaxime 30 µg, imipenem 10 µg, tetracycline 30 µg, penicillin 10 IU, oxacillin 1 µg, vancomycin 30 µg, ceftriaxone 30 µg, and ticarcillin75 µg.

This study has been endorsed by the Ethics Committee of the hospital and the university. Written informed consent was obtained from all patients and the research and ethic committee of TUMS approved the study protocol on human subject.

Statistical Package for Social Sciences were used to analyze data of the current study with employment of student *t*-test for quantitative and Chi square test for qualitative variables while the values were considered statistically significant at a $P<0.05$.

Result

From the 1851 samples, 1257 (68%) were female, also 1666 (90%) were more than 12 year old and 1041 (56.2%) related to hospitalized patients (Table 1).

Table 1-Age & Sex distribution of patients

Age (yr)	Male (594)	Female (1257)
<12	70 (37.8%)	115 (62.2%)
>12	524 (31.5%)	1142 (68.5%)

The main agents of UTI were isolated from outpatients were *E. coli* (51.5%), *S. hemolyticus* and *S. aureus*, and from inpatient, *E. coli* (58%), *Candida* and *Entrobacter* were most common organism (Table 2).

The most common isolated organism in patients below 12 year were *E. coli*, *Entrobacter,* coagulase negative*, Staphylocuccus* and *Proteous* and in above 12 years were *E. coli, Staphylocuccu* coagulase negative and *Candida*. Similarly, *E. coli, Staphylocuccu* coagulase negative and *Candida* were predominant agents in both sexes. In hospitalized patients, *E. coli* was predominant

in Renal Transplant (44.4%), Pediatric (43.3%), Dermatology (51.9%), Urology (41%), Cardiac (53.8%), Emergency (52.2, Internal (43.2%), Surgery (45.7%), Infections (46.3%), Neonate (78.1%) and Gynecology (47.4%) wards. In ICU, the predominant agent was *Candida*, in BMT section was *Acintobacter* and in Neurology section was *Entrococus*.

The antimicrobial resistance pattern of main bacteria is showed in Table 3. Table 4 shows different antimicrobial resistance of *E. coli* in hospitalized patients and outpatients.

Table 2- Distribution of isolated bacteria from inpatients & outpatients

Bactria	Out patient (isolated Bactria %)	Hospitalized Patient (Isolated Bactria %)
E. coli	51.5	58
Candida	3	12
Entrobacter	2	8
Staphylococcus hemolyticus	0.5	5
Staphylococcus aureus	3	5
Others	33	21

Table 3- The antimicrobial resistance pattern of main bacteria

Microbial Agent / Antibiotic	*E. coli (%)*	*Klebsiella(%)*	*Entrobacter(%)*	*Entococuss(%)*	*Pseudomonas(%)*	*Acintobacter(%)*	*Streptococcus nonhemolyticus(%)*	*Protcous(%)*	*Staphylococus Epidermidis(%)*	*Staphylococus hemolyticus(%)*	*Staphylococus saprophyticus(%)*	*Staphylococus areous(%)*	*Citrobacter(%)*
Imipenem	sen 98	-	-	-	Sen* 95	-	-	Sen 94	Res** 100	-	-	Res 66.6	-
Nitroforantoin	Sen 95.5	-	Sen 82.3	-	Res 83.4	-	-	Sen 83.3	-	Sen 9.6	-	-	-
Amikacin	Sen 94.1	Sen 92	Sen 86.7	-	Sen 88	-	-	-	Sen 96	-	-	-	Sen 83.3
Nalidixic acid	Res 62.1	-	-	-	-	-	-	Res 66.6	-	-	-	-	-
Gentamycin	-	Sen 92.5	-	-	Sen 81.4	Sen 94.4	Res 55	-	-	-	-	Sen 99.8	-
Vancomycin	-	-	-	-	-	-	-	-	Sen 98	-	-	-	-
Ampicilin	Res 60	Res 72	Res 69	-	-	-	Sen 90	Res 66.6	-	-	-	-	Res 60
Ceftazidim	Res 87	Res 77	Res 96	-	Res 90	-	Res 60	Res 75	Res 72	Res 100	-	Res 75	Res 100
Ceftriaxon	-	-	-	-	-	-	-	-	Sen 94	Res 71	-	-	-
Penicilin	Res 66.6	-	-	-	-	-	-	-	-	-	-	-	-
Co- trimoxazde	-	-	-	-	Res 95		--	-	-	-	-	Res 48.9	-
Erythromycin	-	-	-	-	-	-	-	-	-	-	Res 100	-	-
Ciprofluoxacin	-	-	-	-	-	-	-	-	-	-	sen	-	-

*: Sensitive **: Resistant

Table 4- Different *E. coli* antimicrobial resistance in hospitalized and out patients

***E. coli* Resistance to**	**Outpatient (%)**	**Hospitalized patient (%)**
Ampicilin	81.6	50.4
Gentamycin	15.7	26.7
Nalidixic acid	40	76.8
Nitroforantoin	3.7	6.8
Amikacin	4.7	6.9
Ceftriaxon	37.7	71.4
Ciprofluoxacin	30.8	46.9
Ceftazidim	30.6	91.2
Co- trimoxazole	47.4	67.6
Imipenem	0.5	2.2

Discussion

This study evaluated species distribution and antibiotic susceptibility of urinary tract infection (UTI) isolated in Imam Hospital of Tehran, Iran. *E. coli* was the most frequent uropathogen (being implicated in more than one half of all the UTI). Similar frequency of isolates of *E. coli* has been obtained in studies performed in Latin American (52%), Norway (56.7%), great Britain (65.1%) and USA (68%) (1, 9-14).

Although *E. coli* was the most common uropathogen in both sexes, its incidence was significantly higher in woman (P=0.0006). The question of an underlying urinary pathology of infection in female as compared to male cases was beyond the scope of this study.

Pseudomonas aeruginosa is most frequent in male due to particular characteristic inherent to the patient including sex, use of antimicrobial agent, previous intervention in urinary tract and patient with neurogenic bladder, but in this study uropathogens were similar in both sexes (1, 3, 14-16).

In this study it was not observed significant difference among the bacteria responsible for these infections in the different age groups.

In outpatients, after *E. coli*, *S. hemolyticus* and *S. areous* were common uropathogen but in hospitalized patients, *Entrobacter* and *Candida* was common pathogen due to more invasive intervention on urinary tract. This finding correlates with previous studies in Norway and Iran (2, 13).

In most wards of the hospital, *E. coli* was most common isolated uropathogen but in some ward other species were isolated.

Although *E. coli* was responsible for more than half of the UTI, its antimicrobial resistance was significantly lower than that presented by the other bacteria (1, 2, 16). In this study, *E. coli* had the most resistance to penicillin, ampicilin, nalidixic acid and Co-trimoxazole but most sensitivity to imipenem, nitroforantoin and amikacin is reported. This finding is compatible with study of Keah with high resistant *E. coli* to ampicilin (63%) and cotrimoxazole (43%) (6).-Also Peterson J study shows resistance to ampicilin (50.1%) and cotrimoxazole (22.1%) (10). The result antimicrobial resistance indicate that cotrimoxazole, nalidixic acid and ampicilin should be ignored to treat of UTI as the resistance rate was higher than the recommended value (<20%) indicated (1, 2, 17-20).

The drugs, once effective in treatment of patients with urinary tract infection, have now become resistant and their effectiveness has declined. Comparison our findings with those from other studies showed that the drug resistance of bacteria causing urinary tract infections in this study is much higher than in most parts of the world

and the drugs prescribed for these infections are ineffective.

While these drugs can still be effective in some parts of the world, this study is recommended to be conducted in every region and every several years so that more appropriate decisions could be made for the treatment of these patients before antibiogram testing.

Besides, when antibiotic resistance is studied, such factors as anomalies in the urinary system, urinary reflux, recent administration of antibiotics and recurrent urinary infection must be taken into account because some of these factors are likely to cause serious problems.

As expected, hospitalized patients have high rate of resistance due to more Antibiotic consumption and more unusual organism due to invasive intervention compared with out patients and attention to antibiogram resistance pattern could be helpful (15,16).

Conclusion

The most common isolated pathogen was *E. coli*. According to antibiogram susceptibility, the recommended antimicrobial drugs are nitroforantoin and imipenem. nalidixic acid and cotrimoxazole are not recommended because drug resistance is high. Antibiotic resistance in this study was remarkable and it must be taken into account. Any unnecessary prescription of antibiotics for patients must be avoided.

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