Original article

Antimicrobial susceptibility patterns of community-acquired uropathogens in Tehran, Iran

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Abstract

Background: Antibiotic resistance of urinary tract pathogens has increased worldwide. Knowledge of the antibiotic resistance patterns of uropathogens in specific geographical locations is an important factor for choosing an appropriate empirical antimicrobial treatment. The aim of this study was to provide information regarding local resistance patterns of urinary pathogens to the commonly used antibiotics in Tehran, Iran.

Methodology: Urine samples collected and submitted to two pathobiology laboratories in Tehran were identified by conventional methods over a period of three years (December 2006 to May 2009). Antimicrobial resistance testing was performed by the standard disk diffusion technique in accordance with the recommendations of the Clinical and Laboratory Standards Institute.

Results: Of the total 13,333 mid-stream urine samples collected from suspected cases of urinary tract infection, 840 (6.3%) were positive for pathogenic bacteria. *Escherichia coli* (*E. coli*) was the most common isolate (68.8%) followed by *Proteus* spp. (12.4%), and *Klebsiella* spp. (9.6%). *E. coli* isolates were mostly susceptible to nitrofurantoin (71.3%), followed by ciprofloxacin (68.1%); however, only 38.2% of *E. coli* isolates were susceptible to trimethoprim-sulfamethoxazole.

Conclusion: Nitrofurantoin may be considered as a first-line empiric antibacterial agent for urinary tract infections in outpatients in Tehran, Iran.

Key words: urinary tract infection, antimicrobial susceptibility, uropathogens

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Introduction

Urinary tract infection (UTI) is not only a common outpatient affliction, but also the most frequently occurring nosocomial infection [1,2]. UTI can be classified into uncomplicated and complicated infections with respect to choices for treatment [1]. Among both outpatients and inpatients, *Escherichia coli* (*E. coli*) is the most common etiological agent, accounting for 75% to 90% of uncomplicated UTI isolates [3,4], while complicated UTI exhibit a broader bacterial spectrum as the cause of infection [1].

The incidence of UTI is greater in women as compared to men, which may be either due to anatomical predisposition or urothelial mucosa adherence to the mucopolysaccharide lining or other host factors [5]. Sexual activity, pregnancy, and obstruction also increase the frequency of UTI [6].

In almost all cases of UTI, empirical antimicrobial treatment initiates before the laboratory results of urine culture are available; thus antibiotic resistance may increase in uropathogens due to frequent misuse of antibiotics [7,8]. For this reason, knowledge of the etiological agents of UTIs and their antimicrobial resistance patterns in specific geographical locations may aid clinicians in choosing the appropriate antimicrobial empirical treatment. Our study was conducted to determine the antimicrobial susceptibility patterns of commonly used antibiotics among community-acquired uropathogens during a three-year period from 2006 to 2009.

Materials and methods

This study was approved by the Institutional Review Board of the University of Tehran.

The study was conducted in two general laboratories that serve outpatients in the western and central parts of Tehran. The study population consisted of all patients having positive community-acquired urine cultures with a colony count of $\geq 10^5$ CFU/mL. Community acquired isolates were defined as a culture collection from a patient not admitted to the hospital. The study was retrospective with an observation period of three years (December 2006 to May 2009). Data on age, sex, result of urine culture, etiological agent, and susceptibility pattern were obtained from the medical records of patients.

A total of 13,333 midstream urine samples were collected in sterile containers from suspected cases of urinary tract infections in two general laboratories (Danesh and Ferdous Pathobiology Laboratories). Each adult patient was carefully instructed regarding the collection of a mid-stream urine sample. Urine samples were obtained by sterile urine bags in infants after disinfecting the perineum.

Urine culture was done using a calibrated loop. Samples were inoculated on blood agar and eosin methylene blue agar plates then were read after overnight incubation at 37° C. For this study, significant bacteriuria was defined as culture of a single bacterial species from the urine sample at a concentration of 10^5 CFU/mL associated with microscope findings of > 10 WBC per high power field [9]. Such urine samples were further processed for identification and antibacterial susceptibility of the uropathogen. When the count was less than 10^5 CFU/ml, it was considered as non-significant bacteriuria or negative.

Identification of bacterial pathogens was made on the basis of Gram reaction, morphology, and biochemical features. All culture media were purchased from Merck, Germany.

Isolates were tested for antimicrobial susceptibility by the standard disk diffusion method according to Bauer *et al*. [10]. Mueller-Hinton agar plates were incubated for 24 hours after inoculation with organisms and placement of the disks and inhibition zones were measured. Antibiotic disks were obtained from Padtanteb Company, Iran. The commercial antibiotics used for isolates included ciprofloxacin (CP), trimethoprim-sulfamethoxazole (SXT), gentamicin (GM), ampicillin (AM), nitrofurantoin (FM), nalidixic acid (NA), Ceftriaxone (CRO), ceftizoxime (CT), cephalexin (CN), cephalothin (CF), amoxicillin (AMX), carbenicillin (CB), norfloxacin (NOR), ceftazidime (CAZ), penicillin (P), erythromycin (E), and vancomycin (V); P, E, and V were used for gram positive bacteria. The results were interpreted according to CLSI (formerly NCCLS) 2000 [11].

Statistical analysis was performed using SPSS, version 13 (SPSS Inc., Chicago, IL, USA).

Results

A total of 13,333 urine samples were analyzed for isolation and identification of bacterial isolates. Of these, 840 (6.3%) samples were found to be significant bacteriuria and the remaining 12,493 samples were either non-significant bacteriuria or had a very low bacterial count or were sterile urine.

Among the all patients, 85.2% were female. Ages ranged from one to 94 years with an average age of 48.5 (SD = 21.8) years. The mean age of male and female patients was 53.5 (SD = 20.8) years and 47.6 (SD = 21.8) years, respectively.

Table 1 illustrates the overall frequency and rank order of community-acquired uropathogens. As expected, *E. coli* was the most frequently reported isolate (68.8%).

Gram-positive organisms included only 5.3% of the isolates in our study.

Antimicrobial susceptibility results are summarized in Table 2. *E. coli* isolates were mostly susceptible to nitrofurantoin (71.3%), followed by ciprofloxacin (68.1%). *E. coli* isolates had the highest resistance rate to ampicillin and amoxicillin (85.9% and 95.2%, respectively). Importantly, only 38.2% of *E. coli* isolates were susceptible to trimethoprim-sulfamethoxazole. *Proteus* spp. showed the highest sensitivity to ciprofloxacin (71.2%) and the highest resistance to ampicillin (88.3%) and to cephalothin (58.3%). *Klebsiella* spp. had the highest sensitivity to ciprofloxacin (81.3%) and showed the highest resistance rate to ampicillin and amoxicillin (94.5% and 88.5%, respectively).

Pseudomonas, which has a high resistance rate worldwide, was 100% resistant to ampicillin, 92.9% to nalidixic acid and 88.9% to nitrofurantoin. The best activity against *Pseudomonas* (75% susceptible) was attained with ciprofloxacin.

Table 1. Frequency of community-acquired uropathogens.

Organism	No. of isolates (%)
E. coli	578 (68.8)
Proteus spp.	104 (12.4)
Klebsiella spp.	81 (9.6)
Pseudomonas spp.	28 (3.3)
Streptococcus spp. [1]	19 (2.3)
Enterococcus spp.	11 (1.3)
Coagulase-Negative Staphylococci [2]	9 (1.1)
Staphylococcus aureus	5 (0.6)
Enterobacter spp.	3 (0.4)
Citrobacter spp.	2 (0.2)

[1] includes non-hemolytic streptococci (1), α-hemolytic streptococci (2), and β-hemolytic streptococci (16)
[2] includes *S. saprophyticus* (4) and *S. epidermidis* (5)

Table 2. Antimicrobial susceptibility among community-acquired uropathogens. Note that intermediate categories are not mentioned.

	E. coli (578)		*Proteus* spp. (104)		*Klebsiella* spp. (81)		*Pseudomonas* spp. (28)		*Streptococccus* spp. (19)		*Enterococcus* spp. (11)		CoNS (9)		*S. aureus* (5)		*Enterobacter* spp. (3)		*Citrobacter* spp. (2)	
	#T[1]	%S[2]	#T	%S	#T	%S	#T	%S	#T	%S	#T	%S	#T	%S	#T	%S	#T	%S	#T	%S
Ciprofloxacin	564	68.1	104	71.2	80	81.3	28	75	-	-	-	-	-	-	-	-	3	100	2	100
Norfloxacin	234	62	-	-	24	91.7	5	80	15	86.7	10	60	9	88.9	4	75	2	100	1	100
Nalidixic acid	577	30.5	104	10.6	81	43.2	28	3.6	15	6.7	10	10	9	0	4	0	3	66.7	2	100
Gentamicin	578	49.3	104	7.7	81	53.1	28	21.4	15	0	11	18.2	9	88.9	4	100	3	33.3	2	100
Nitrofurantoin	575	71.3	104	42.3	81	27.2	27	3.7	14	100	11	90.9	9	88.9	4	75	3	33.3	2	100
Penicillin	-	-	-	-	-	-	-	-	13	7.7	11	0	8	12.5	4	0	-	-	-	-
Ampicillin	304	3.6	103	1	55	0	22	0	-	-	-	-	-	-	-	-	-	-	-	-
Amoxicillin	271	2.6	-	-	26	11.5	6	0	15	93.3	11	45.5	8	25	4	75	3	33.3	2	50
Carbenicillin	269	72.9	-	-	26	96.2	6	50	-	-	-	-	-	-	-	-	3	100	2	100
Cephalexin	304	11.5	103	2.9	55	9.1	22	4.5	-	-	-	-	-	-	-	-	-	-	-	-
Cephalothin	304	15.1	103	1.9	55	12.7	22	4.5	-	-	-	-	-	-	-	-	-	-	-	-
Ceftazidime	247	62.8	-	-	23	82.6	6	83.3	-	-	-	-	-	-	-	-	2	100	2	100
Ceftriaxone	304	63.5	103	40.8	55	47.3	22	9.1	-	-	-	-	-	-	-	-	-	-	-	-
Ceftizoxime	304	64.1	103	39.8	55	61	22	13.6	-	-	-	-	-	-	-	-	-	-	-	-
Trimethoprim-sulfamethoxazole	578	38.2	104	22.1	81	46.9	28	10.7	15	0	11	18.2	9	88.9	4	75	3	100	2	100
Erythromycin	-	-	-	-	-	-	-	-	14	64.3	10	10	9	77.8	3	66.7	-	-	-	-
Vancomycin	-	-	-	-	-	-	-	-	14	85.7	11	18.2	9	66.7	4	100	-	-	-	-

1) Number of isolates tested against each antimicrobial agent
2) Percent of isolates susceptible to antimicrobial agent

Discussion

The worldwide trend of empirically treating community acquired UTI may not apply to specific geographical regions such as Iran, where decreased susceptibility rates are documented for common urinary pathogens [12,23-25]. Therefore, it is important to monitor the status of antimicrobial resistance among uropathogens to improve treatment recommendations. We conducted this study to determine the frequency and antimicrobial susceptibility patterns of community-acquired uropathogens in our region.

Our study was retrospective using the results of routine diagnostic and susceptibility analysis in two general laboratories of Tehran, Iran. Our data was restricted to patients who can afford medical analysis; therefore, this study may not reflect the true prevalence of UTI among patients in Tehran as most patients are initially treated empirically for their UTI. Also, susceptibility testing was not complete for all antimicrobials used to treat UTIs caused by uropathogens.

In our study, as in several previous reports, the most commonly isolated organism in UTI was *E. coli*, involving 68.8% of the positive samples [12-14]. The proportion of bacterial species isolated was similar to those described in several previous studies [12,13,15,16]. *Proteus* spp. was the second most common organism followed by *Klebsiella* spp. and *Pseudomonas* spp.

The frequency of UTI is greater in women as compared to men [5,13], and our results were similar to these reports; 85.2% of all patients were female.

Trimethoprim-sulfamethoxazole, fluoroquinolone, or nitrofurantoin are recommended for empirical treatment of uncomplicated UTI [17,18]. However, studies from the United States of America and worldwide indicate the emergence of high levels of trimethoprim-sulfamethoxazole resistance in a significant percentage (> 20%) of community-acquired *E. coli* UTI isolates [19-22]. We also found a high level of resistance to this antimicrobial agent [54%]. Other Iranian studies reported similar results [23,24]. These findings indicate that initial empirical treatment with trimethoprim-sulfamethoxazole is no longer appropriate in Tehran.

The fluoroquinolones tested in this study (ciprofloxacin and norfloxacin) show relatively good activity against *E. coli*, finding that 68.1% and 62% of the *E. coli* strains were susceptible to ciprofloxacin and norfloxacin, respectively. This result, which was lower than that obtained by another Iranian study [25], may be due to a shift in antibiotic prescription toward fluoroquinolones in recent years in Iran. These findings indicate that the empiric use of fluoroquinolones should be seriously reconsidered in our region, or that strategies to counteract increased resistance to these antibiotics must be developed.

Nitrofurantoin demonstrated better activity against *E. coli* isolates (71.3% susceptible), but this drug would not be recommended for serious upper urinary tract infections or for those cases with systemic involvement [26].

According to a Turkish study [27], *E. coli* isolates were highly resistant to ampicillin (47.8% to 64.6%) and higher resistance rates to ampicillin have been reported in other countries including Senegal (77%), Spain (65%), Taiwan (80%), and India (88%) [28-31]. In our study, the ampicillin resistance rate was 88%. The beta (β)-lactam antibiotics such as ampicillin have other problems besides resistance. They are found to have relatively poor action in treating symptomatic cystitis. One hypothesis is that it is rapidly excreted and the duration of significant drug concentration in the urine is short. The other reason is that β-lactams are relatively ineffective in clearing Gram-negative rods from the vaginal and colonic mucosa, thus possibly predisposing to recurrences when used to treat UTI (18,32).

In conclusion, trimethoprim-sulfamethoxazole is not recommended as a first choice for treatment of UTI in Tehran area. Nitrofurantoin may be considered as a first-line empiric agent in outpatients. As resistance to fluoroquinolones is increasing in the community for *E. coli*, severely curtailing fluoroquinolone use in uncomplicated infections is recommended.

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