Original Article

A Survey on Urinary Pathogens and their Antimicrobial Susceptibility among Patients with Significant Bacteriuria

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ABSTRACT

Background and Objective: **Urinary tract infection (UTI) is one of the most common diagnosed infectious diseases. In order to determine the spectrum of bacterial etiology and antibiotic resistance pattern of the uropathogens that cause community acquired UTI, a retrospective study was performed in Research Center of Reference laboratories of Iran on urine samples of referred patients during a 4 years period (from 2002 to 2006).**

Materials and Methods: **During the study period, 4207 urine specimens were cultured. Cultures with a significant bacterial growth were selected and susceptibility testing was performed using disk diffusion method.**

Results: **Out of 4207 urine specimens, 476 (11%) yielded positive culture. *Escherichia coli* (44.5 %) and *Klebsiella pneumoniae* (8.5%) were the most predominant isolated bacteria respectively. *Escherichia coli* revealed the highest susceptibility among oral antibiotics to nitrofurantoin (76%) and the highest rate of resistance to carbenicillin (94%) and ampicillin (89%) respectively. In total isolates, the highest level of susceptibility was for imipenem (99%) and among oral antibiotics for nitrofurantoin (70 %), and the highest level of resistance was for carbenicillin (89%) and ampicillin (84%).**

Conclusion: **The results of this study revealed a considerable resistance to fluoroquinolones and cotrimoxazole and a high sensitivity to nitrofurantoin among isolated bacteria. We strongly recommend nitrofurantoin as the drug of choice for empiric therapy of UTI in our country.**

Key words: Urinary tract infections, Community-Acquired Infections, Microbial Sensitivity Tests

Introduction

Urinary tract infections (UTIs) are among the most common infectious diseases in outpatients as well as in hospitalized patients and can lead to significant mortality. There are an estimated 150 million urinary tract infections per annum worldwide (1-3). It accounts for a large proportion of antibiotic consumption and has a large socio-economic impact and may contribute to the emergence of bacterial resistance (4). In recent years, attempts

Received: 7 April 2008
Accepted: 6 September 2008
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to manage reimbursable medical costs have led to a decrease in physician orders for routine urine cultures and subsequent susceptibility testing in symptomatic patients and treatments begin or are done completely empirically (3). Antimicrobial resistance among uropathogens causing community acquired UTIs, both cystitis and pyelonephlitis, is increasing and a growing problem and urinary tract infections have become more complicated and difficult to treat recently (5;6).

The prevalence of etiologic agents and the pattern of their resistance to antibiotics is changing, so it is very important to assist general practitioners to select the most cost-effective antibiotics with the fewest adverse reactions for empiric therapy.

Therefore, this study was designed to assess the prevalence of urinary pathogens and their susceptibility pattern to different currently used antibiotics in outpatients during a period of four years in Research Center of Reference Laboratories of Iran (Reflabs).

Materials and Methods

In this retrospective study, the urine specimen of 4207 patients between 10 and 80 years of age with or without the symptoms of lower UTI referred to Research Center of Reference Laboratories of Iran (Reflabs) were examined. Out of 4207 patients, 476 cases (143 males and 333 females) revealed significant bacteriuria ($>10^4$ CFU/ml). The exclusion criteria were: inadequate urine sample (less than 10 ml urine submitted), urine bag collected specimens, the specimens collected more than 2 hrs before submitting, specimens submitted in leaking or dirty unsuitable containers, specimens collected in inappropriate sampling manner (not clean catch), and specimens revealing growth of more than 2 types of bacteriae in their culture plate

Urine sampling procedure

Patients were requested to provide a freshly voided midstream clean urine specimen in a sterile wide mouth container which the lab provided according to guidelines of admission personnel.

Identification

Upon receipt of specimen, within a maximum of one hour, the urine sample was cultured on blood agar and MacConkey plates separately. All plates were incubated at 37 ºC for 24 hours. All bacteria growing on the culture media at 10^4 colony forming units (CFU)/ml were identified by their biochemical reaction profile using Beckton & Dickinson and Mast Diagnostic Group UK identification products. In our study, we also accepted mixed cultures which do not reveal more than two predominant bacteriae. We categorized isolated bacteria into four groups including enterobacteriaceae, non-enterobacteriaceae gram negative organisms, *Staphylococcus spp*, and *Streptococcus spp.*

Antimicrobial susceptibility testing

The isolated bacteria were inoculated on Mueller Hinton agar (Mast group Ltd, Merseyside, UK) and antimicrobial susceptibility testing was performed using disk diffusion method (as recommended by CLSI No: M2-A9) (7). The antibiotic disks were provided from Mast diagnostic group Ltd. The antibiotic panels for each group of isolates were selected according to CLSI guidelines (as described by Clinical and Laboratory Standard Institute (CLSI) No: M100-S16) (8). The inhibition zone diameter was measured using a scaled ruler (antibiotic zone scale) and reported as resistant, intermediate and susceptible. *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, cephalothin 30 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 ticarcillin 75 µg.

Results

A total number of 4207 urine samples from patients with or without lower UTI symptoms referred to Reflabs during four years (2002-2006) were included in our study. Among these samples, the culture

revealed positive result in 476 cases (11%). Out of 476 patients, 333 (76%) cases were female and the female to male ratio was 2.2. The mean age of the study population was 37 years old .Among the isolated strains, 63% of them were gram negative bacilli and 59% of them were enterobacteriaceae. The frequency of other isolated pathogens was 22%, 15% and 4% for *Non-enterococcal streptococci, Staphylococcus spp and Enterococcus* spp respectively. In the *Enterobacteriaceae* group, the frequency of *E. coli* and *Klebsiella pneumoniae* were 75% and 14% respectively. Distribution of other species in this group is shown in Table 1. In addition, four percent of total isolates was non-enterobacteriaceae gram-negative organisms, among them 67% was *Pseudomonas aeruginosa* and 34% was *Acinetobacter beumanii* as is shown in Table 2. The distribution of isolated species of *Streptococccus* is shown in Table 3. Among isolated Streptococcus group, the frequency of *Streptococcus group B and D* was 31% and 24% respectively. Table 4 reveals distribution of *Staphyloccocus spp.* Out of all isolated bacteria, the highest level of susceptibility was for imipenem (99%), amikacin (84%), vancomycin (100%), nitrofurantoin (70%) and gentamicin (67%). The highest level of resistance was for carbenicillin (89%), ampicillin (84%), cefalothin (80%), oxacillin (75%) and trimetoprim-sulfamethoxazole (72%). Among these isolates, *E. coli* showed the highest susceptibility to imipenem (99%), amikacin (95%) and nitrofurantoin (75%) and *Klebsiella pneumoniae* showed the highest susceptibility to imipenem (100%), amikacin (70%), gentamicin (64%) and cefotaxime (56%). Susceptibility patterns of most frequent uropathogens to different antibiotics are shown in Table 5.

Table 1: Distribution of isolated species of enterobacteriaceae

Genus/Species	percent in group	percent in total
E. coli	74.9	44.54
Klebsiella pneumonia	14.14	8.4
Klebsiella oxytoca	0.71	0.42
Citrobacter diversus	1.06	0.63
Citrobacter freundii	2.47	1.47
Citrobacter farmer	0.35	0.21
Enterobacter aerogenesis	1.06	0.63
Enterobacter cloacae	2.47	1.47
Proteus mirabilis	0.71	0.42
Proteus vulgaris	0.71	0.42
Serratia marcesence	1.41	0.84
Total	100	100

Table 2: Distribution of isolated species of non-enterobacteriaceae gram-negative rods

Genus/Species	Percent in group	Percent in total
Pseudomonas aeroginosa	66.67	2.52
Acinetobacter beumanii	33.33	1.26
Total	100	3.78

Table 3: Distribution of isolated species of *Streptococcus spp*.

Genus /Species	Percent in group	Percent in total
Streptococcus type A	3.88	0.84
Streptococcus type B	31.07	6.72
Streptoccocus type D	24.27	5.25
Streptococcus viridians	22.33	4.83
Enterococcus	18.45	4
Total	100	21.64

Table 4: Distribution of isolated species of *Staphylococcus spp*.

Staphylococcus aureus	**Percent in group**	**Percent in total**
Staphylococcus haemolyticus	33.32	5.04
Staphylococcus epidermidis	40.28	6.1
Staphylococcus saprophyticus	12.5	1.89
Total	100	15.04

Table 5: Sensitivity (%susceptible) of the most frequently isolated bacteria

Antibiotic	*E. coli*	*Klebsiella*	*Streptococcus spp*
Ampicillin	11	13	52
Nitrofurantoin	76	31	70
Gentamycin	68	64	25
Amikacin	95	70	-
Cefotaxim	66	56	-
Imipenem	99	100	-
Penicillin	-	-	64
Cephalothin	-	-	81
Ceftriaxon	-	-	53
Vancomycin	-	-	83

Discussion

Regarding the important role of periodical surveillance of bacterial etiology and antibiotic resistance in health care units and the importance of guiding general practitioners about locally prevalent strains and their susceptibility pattern, we decided to study urine cultures in a four year period and to determine antimicrobial susceptibility profile. Our study is retrospective using the results of our routine

diagnostic and susceptibility analysis in research center of Reference laboratories of Iran.

Urinary tract infection occurs in every age and in both genders, but it is more frequent in females. In our study, the most frequent uropathogen in both genders was enterobacteriaceae (60%) with a female to male ratio of 2.5. In general, the most frequent uropathogens in both genders were *E. coli* and *Klebsiella pneumoniae* with a frequency of 44.5% and 8.5% respectively which in comparison with three other studies that have been performed in Iran (9), Norway (4) and Kuwait (10), similar results were obtained. *E. coli* as the most prevalent uropathogen revealed the highest susceptibility among oral antibiotics to nitrofurantoin (76%), similar to the result of a study performed in Brazil (11) which it was 77%. The highest rate of resistance among all isolates was for carbenicillin (94%) and ampicillin (89%), similar to the result of another Iranian study (9).

In this study, *E. coli* in 60% of cases was resistant to fluoroquinolones which was higher than other studies carried out in Iran (9) and Okada (12). In some studies, fluoroquinolone regimens seem superior to SXT for empiric therapy because of the relatively high prevalence of SXT resistance among uropathogens causing pyelonephritis (13). As mentioned by Kahlmeter, incresaing quinolone resistance in community for *E. coli*, primarily in Spain and Portugal, is a cause for immediate action, one of which to severely curtail quinolone use in uncomplicated infections (14). It seems that the mentioned difference in fluoroquinolone resistance in our study and another one (5) is due to a shift in antibiotic prescription toward fluoroquinolone in recent years in Iran. As the study conducted by Karlowsky et al, ciprofloxacin is the only agent demonstrated a stepwise, >3-fold increase in resistance from 1995 (0.7%) to 2001 (2.5%). Given that fluoroquinolone resistance among gram negative bacteria is found predominantly among multidrug resistant isolates, it suggests that fluoroquinolone resistance will perhaps accelerate even if other antimicrobials are used (3).

In our study, 74% out of isolated *E. coli* were resistant to co- trimoxazole (similar to the other Iranian study) (9) and one of them had intermediate susceptibility to above mentioned antibiotic. Treatment guidelines recommend co-trimoxazole for empiric therapy of uncomplicated UTI, unless co-trimoxazole resistance in a community exceeds 10-20% (3). Therefore, we do not recommend SXT as a first choice for treatment of UTI as an empiric therapy in Iran. Use of nitrofurantoin for the empiric treatment of mild cystitis is supportable from a public health perspective in an attempt to decrease uropathogen resistance because it does not share cross-resistance with more commonly prescribed antimicrobials (13).

Conclusion

Since UTI has a large socio economic impact and may contribute to the emergence of bacterial resistance, the following subjects are strongly recommended: periodic surveillance of antibiotic susceptibility in a systematic manner under supervision of a joint scientific committee of scientific experts and physicians and according to our results that revealed a high sensitivity of most frequent uropathogens to nitrofurantoin, it is tempting to assume that the change in consumption of nitrofurantoin is the cause of increased bacterial sensitivity, so this antibiotic may be considered as the first choice for empiric therapy in outpatients.

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