Iranian Journal of Pharmaceutical Sciences
Summer 2009: 5(3): 135-140
ijps.sums.ac.ir

Original Article

Comparison of Antibiotic Sensitivity of Prevalent Bacteria Isolated from Urinary Tract Infections in 2002 and 2006 in Urmia, Iran

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Abstract

Urine specimens collected from in-patients and out-patients in Urmia Imam Hospital, northwest Iran, were cultured on blood agar and Eosin Methylene Blue agar. Isolated bacteria were identified according to standard microbiological tests and then subjected to sensitivity testing according to routine method of disk agar diffusion technique. Out of 8044 and 10425 urine specimens, 8.7% and 11.9% were identified as having urinary tract infection (UTI) in 2002 and 2006, respectively. The most prevalent bacteria belonged to enterobacteriaceae family and in the case of total susceptibility the upmost resistance was recorded against trimethoprim-sulfamethoxazole (62%) and gentamicin (50%) in 2002, and increased to 69% and 57% in 2006, respectively. The least resistance recorded was to ceftizoxim as 15.6% and 16.8 % in 2002 and 2006, respectively. Antibiotics susceptibility of in-patients was significantly lower than that of out-patients and this was more obvious for cephalosporins. Our findings show a remarkably high prevalence of resistance to the majority of commonly used antibiotics in UTIs, with a decreasing trend in their activities which probably is due to the high rate of antibiotics use in Iran as the first reason. Results of the present study underline the need for sensitivity tests prior to antibiotic therapy in UTI, which could help and guide in proper choosing of antibiotics and effective treatment and, therefore, prevention of antibiotic resistance.

Keywords: Antibiotic resistance; Antimicrobial susceptibility; Urinary tract infection.
Received: December 7, 2008; *Accepted:* April 6, 2009

1. Introduction

Urinary tract infection (UTI) is one of the most common infections that afflicts humans and if not treated properly and on time could cause serious damages to the urinary tract [1]. At least 150 million cases are diagnosed with UTI annually, putting a fortune on global economy [2]. *Escherichia coli* is the most

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frequent bacteria isolated from UTI with more than 75% prevalence. Nowadays, increase of antibiotic resistance is one of the problems in managing of UTI [1-3]. Availability of new information about sensitivity of prevalent bacteria in a given area could help in the selection of proper treatment regimens especially in empirical therapy which is naturally based on such information. The objective of the present study was to compare the prevalence of causative bacteria and antibiotics susceptibility patterns of the bacteria isolated from urine samples of in-patients and out-patients in a 6 months periods in 2002 and 2006 to compare them.

2. Materials and methods

2.1. Sensitivity study method

Urine specimens of in-patients and out-patients were retrospectively studied for causative agent and antibiotic susceptibility patterns in Urmia Imam Hospital, Urmia, Iran, in 6 months periods during 2002 and 2006. All specimens used was cultured on blood agar, eosin methylene blue agar (EMB), and if positive were tested on other differential and diagnostic culture media such as manitol salt agar, simon citrate, SIM, MR/VP, TSI and other differential media (Hi media). Isolated bacteria were identified according to conventional microbiological tests such as gram staining and biochemical tests like catalas, coagulase, indole, urease, carbohydrate fermentation and then subjected to sensitivity testing according to routine method of disk agar diffusion technique [4]. Briefly, single colony from each isolate was transferred into 4 ml broth medium and incubated overnight at 37 °C, then cells were harvested by centrifugation at 3000 rpm for 5 min. and re-suspended in Ringer solution to provide bacterial concentrations of 1.5×10^8 CFU/ml or equivalent to 0.5 McFarland. The surface of a Brain Heart Infusion (BHI) agar inoculated and test antibiotic were placed on the seeded plates and incubated overnight at 37 °C. The diameters of growth inhibition zones were measured and according to the manufacturer guidelines were classified as sensitive (s) or resistant (r). The antibiotic discs used were as follows: Ceftizoxim (CT, 30 μg), ceftriaxon (CRO, 30 μg), norfloxacin (NOR, 10 μg), ciprofloxacin (CP, 5 μg), ceftazidime (CAZ, 30 μg), nitrofurantoin (F/M, 300 μg), nalidixic acid (NA, 30 μg), amikacin (AN, 30 μg), gentamicin (GM, 10 μg), co-trimoxazole (SXT, 25 μg), vancomycin (V, 30 μg), erythromycine (E, 15 μg) and pencillin G (P, 10 μg), (Hi media).

2.2. Statistical analysis

Sensitivity of isolates to various antibiotics was analyzed by one-way ANOVA (SPSS-13 for windows) and differences with $p<0.05$ were considered as significant.

3. Results

A total of 8044 and 10425 urine specimens were tested for bacteria causing UTI in 2002 and 2006, respectively, from which 699 (8.7%) and 1238 (11.9%) cases were recorded as positive for UTI. The most prevalent bacteria belonged to Enterobacteriaceae family. *E. coli* was marked in the first place with 74% total prevalence in both periods and *Staphylococcus* spp. *Pseudomonas aeruginosa* and *Klebseilla pneumonia* gained the next places with 9.6%, 7.7%, and 5%, respectively in 2002, while they were ranked as 8.5%, 4.6% and 8.2%, respectively in 2006. Two time scale variation considering the type of isolates was not significant.

The details of the bacteria isolated from the positive samples are shown separately for in-patients and out-patients in Table 1. The frequency of *E. coli* in in-patients is significantly lower than in out-patients. On the other hand, detection of isolates such as *P. aeruginosa*, *K. pneumonia* and *Staphylococcus* spp. were approximately duplicated in in-patients. Positive urine cultures were recorded as 63% and 59% for females in

Table 1. Frequency of bacterial isolates causing UTI in out-patients and in-patients.

Organism	No.(%)		Female		Male		In-patient		Out-patient	
	2002	2006	2002	2006	2002	2006	2002	2006	2002	2006
Gram-negative organisms	**626**	**1109**	**399**	**918**	**231**	**191**	**408**	**671**	**218**	**427**
E. coli	520 (74.4%)	925 (74.7%)	353 (79.9%)	767 (74.7%)	167 (65%)	158 (74.9%)	337 (76.4%)	527 (70.4%)	183 (70.9%)	332 (67.8%)
P. aeruginosa	54 (7.7%)	57 (4.6%)	19 (4.3%)	46 (4.5%)	35 (13.6%)	11 (5.2%)	35 (8%)	83 (11.1%)	19 (7.4%)	25 (5.1%)
K. pneumonia	35 (5%)	102 (8.2%)	15 (3.4%)	87 (8.5%)	20 (7.8%)	15 (7.1%)	21 (4.8%)	39 (5.2%)	14 (5.4%)	41 (8.4%)
Entrobacter spp.	2 (0.3%)	9 (0.8%)	3 (0.7%)	6 (0.6%)	1 (0.4%)	3 (1.4%)	2 (0.4)	6 (0.8%)	-	9 (1.9%)
Proteus spp.	6 (0.9%)	11 (0.9%)	4 (0.9%)	8 (0.7%)	2 (0.8%)	3 (1.4%)	4 (0.9%)	9 (1.2%)	2 (0.8%)	15 (3.1%)
Acintobacter spp.	9 (1.3%)	5 (0.4%)	5 (1.1%)	4 (0.4%)	6 (2.3%)	1 (0.5%)	9 (2%)	7 (0.9%)	- (1%)	5
Gram-positive organisms	**73**	**129**	**43**	**109**	**26**	**20**	**33**	**78**	**40**	**62**
Staphylococcus spp.	67 (9.6%)	106 (8.5%)	39 (8.8%)	94 (9.1%)	25 (9.7%)	12 (5.7%	29 (6.6%)	67 (8.9%)	38 (14.7%)	53 (10.8%)
Streptococcus spp.	6 (0.8%)	23 (1.9%)	4 (0.9%)	15 (1.5%)	1 (0.4%)	8 (3.8%)	4 (0.9%)	11 (1.5%)	2 (0.8%)	9 (1.9%)
Total	**699**	**1238**	**442**	**1027**	**257**	**211**	**441**	**749**	**258**	**489**

2002 and 2006, respectively, whereas males gained only 37% and 41% of positive cases in the same periods. Bacteria isolated from positive cases are shown in Table 1. Considering the types of isolates shown in Table 1, the prevalence order of isolates were the same in males and female except for *P. aeruginosa* that was in the second position for males while it was in the third place in females. The first rank isolates in both genders were *E. coli.*

The mean percentage of antibiotic susceptibility testing results is summarized in Table 2. The antibiotic susceptibility patterns of isolates from in-patients and out-patients are compared in Table 3. According to the results, overall antibiotics susceptibility of in-patients was significantly lower than that of out-patients, and this is more obvious for cephalosporins with high hospital usages. Based on these results, *E. coli* isolates were more susceptible to the majority of tested antibiotics than *K. pneumonia* and *P. aeruginosa* which showed moderate and weak susceptibility to the tested antibiotics.

As it can be seen, the upmost resistance of *E .coli* was recorded against trimethoprim-sulfamethoxazole and gentamicin (62% and 50%, respectively) in 2002 and increased significantly in 2006 (69% and 57%, respectively), while the least resistance recorded was to ceftizoxim (15.6%, 16.8 % in 2002 and 2006, respectively) which is not significantly different.

In the case of *P. aeruginosa*, nalidixic acid, nitrofurantoin and trimethoprim-sulfamethoxazole showed the least activities (2.6%, 3.8% and 5.2% of susceptibility, respectively) in 2002 and were approximately the same in 2006. Similarly, nitrofurantoin and trimethoprim-sulfamethoxazole exhibited little efrfect against *K. pneumonia* in both studied periods as illustrated in Table 2.

4. Discussion

This study was accomplished to compare the pathogens involved in UTIs in Urmia (northwest, Iran) Imam Hospital in two 6 months periods in 2002 and 2006.

As in many other studies, *E. coli* remained the main cause in UTIs (74% of all isolates), with no significant changes in the distribution of other Enterobacteriaceae. The predominance of *E. coli* among gram-negative bacteria was consistent with many other researches [2, 5-7] and probably related to the

Table 2. Antibiotic susceptibility percentage of common UTI isolates in 2002 and 2006.

Antibiotic	*E. coli*		*P* values*	*P. aeruginosa*		*P* values	*K. pneumonia*		*P* values
	2002 (n=520)	2006 (n=925)		2002 (n=54)	2006 (n=57)		2002 (n=35)	2006 (n=102)	
Amikacin	56.7	50.2	0.001 (S)	41.9	28.5	0.000 (S)	54.5	38.5	0.000 (S)
Ceftazidime	65.4	69.3	0.009 (S)	28.1	19.7	0.003 (S)	52.6	30.7	0.000 (S)
Ceftizoxim	84.4	83.2	0.35 (NS)	8.8	5.6	0.017 (S)	54.1	37.8	0.000 (S)
Ceftriaxon	84.0	78.6	0.006 (S)	5.2	5.3	0.802 (NS)	61.5	42.9	0.000 (S)
Ciprofloxacin	79.2	82.3	0.056 (NS)	42.8	26.7	0.000 (S)	62	59	0.192 (NS)
Co-trimoxazole	37.8	31.4	0.001 (S)	9.7	5.4	0.002 (S)	22.2	17.6	0.007 (S)
Gentamicin	50.9	43.6	0.005 (S)	43.9	23.1	0.000 (S)	40	32.7	0.005 (S)
Nalidixic acid	59.3	62.7	0.023 (S)	2.6	3.5	0.196 (NS)	35.7	31.7	0.008 (S)
Nitrofurantoin	59.4	56.7	0.088 (NS)	3.8	5.3	0.319 (NS)	9.6	10.7	0.077 (NS)
Norfloxacin	81.7	73.8	0.001 (S)	42.3	20.7	0.000 (S)	57.1	23.9	0.000 (S)

*S: significant; NS: non significant

fact that it is a normal flora of the large intestine. Consistent with other studies, the frequencies of UTI positive females were remarkably higher compared to males [3].

Overall susceptibility testing of different antibiotics in this research indicates distressing raising resistance against many commonly used antimicrobial agents from 2002 to 2006. This is in accordance with the global observation of increased resistance to antibiotics but remarkably precedes the average rates reported from other countries [8, 9]. It is generally accepted that selective application of antibiotics is the main risk factor for emergence and dissemination of antibiotic resistance [9]. Set-up and implementation of guidelines regarding optimal antibiotic use based on actual local resistance data is essential to reduce morbidity and mortality [10]. Based on the data, the resistance of isolates from in-patients was significantly higher than from out-patients, in a good agreement with other works [3]. Also, according to the results pertaining to the bacteria type, duplication of isolates such as *P. aeruginosa*, *K. pneumonia* and *Staphylococcus* spp. indicate the presence of nosocomial pathogens among in-patients which is in good agreement with other studies [3].

The relatively high percentage of *P. aeruginosa* resistant to the different cephalosporins tested is probably due to the intrinsic high resistance level of the bacteria [11]. However, *K. pneumonia* and *E. coli* isolates are on the top of susceptibility list to cephalosporins. They also follow a rapid decreasing trend in their susceptibility to cephalosporins, which may be related to some factors, more dominantly high level of cephalosporins used in general practice in recent years in Iran, as well as prevalence of resistant isolates, probably due to the high frequency of ESBL-producing isolates in our hospitals. In a study performed by Mehrgan and coworker, prevalence of extended-spectrum β-lactamase-producing *E. coli* in a tertiary care hospital in Iran has been shown to be 67.2%, indicating a very high incidence of ESBL production by *E. coli* isolates that may have been caused by the excessive use of broad-spectrum antibiotics in the community setting, together with a lack of attention to laboratory screening of ESBL production by clinical isolates [12].

Of the aminoglycosides, amikacin showed more activity than gentamicin against all isolates in accordance with other studies [8-12], however high percentages of resistance were monitored against both agents in our hospital. Aminoglycosides should be used in combination with β-lactam antibiotics to treat serious infections due to ESBL-positive organisms [12].

Among fluoroquinolones, acceptable *in vitro* susceptibility in *E. coli* and *K. pneumonia* isolates has been monitored but rapidly dropped in 2006 and followed the overall trend of other antibiotics. Similar findings have been documented, previously [2].

Table 3. Antibiotic susceptibility percentage of common UTI isolates in out-patients and in-patients.

Antibiotic	*E. coli*				*P. aeruginosa*				*K. pneumonia*			
	2002		2006		2002		2006		2002		2006	
	In-patient	Out-patient	In-patient	Out-patient	In-patient	Out-patient	In-patient	Out-patient	In-patient	Out-patient	In-patient	Out-patient
Amikacin	45.4	68.0	38.4	62.0	41.9	-	28.5	-	46.7	62.3	33.1	43.9
Ceftazidime	59.8	71.0	63.6	75.0	27.9	28.3	18.5	20.9	59.8	45.4	28.7	32.7
Ceftizoxim	80.2	88.6	81.3	85.1	5.2	12.4	2.8	8.4	50.2	58.0	38.8	36.8
Ceftriaxon	73.4	94.6	74.2	83.0	4.6	5.8	5.0	5.6	62.3	60.7	37.5	47.9
Ciprofloxacin	78.3	80.1	80.3	84.3	40.4	45.2	19.3	34.1	62.1	61.9	52.3	65.7
Co-trimoxazole	42.6	33.0	30.5	32.3	8.5	10.9	-	5.4	28.8	22.6	14.5	20.7
Gentamicin	48.4	53.4	36.9	50.3	28	59.8	15.3	30.9	36.2	43.8	41.2	35.8
Nalidixic acid	63.8	54.8	61.9	63.5	0	5.2	2.4	4.6	29.7	46.7	22.7	40.7
Nitrofurantoin	62.4	56.4	55.0	58.4	1.3	6.3	4.6	6.0	5.6	15.3	10.0	11.4
Norfloxacin	79.6	83.8	71.1	76.5	33.8	50.8	10.2	31.2	51.1	63.1	25.0	22.8

Susceptibility of many other commonly used agents like trimethoprim-sulfamethoxazole was low and followed decreasing trend over the examined time period. As for *E. coli* isolates, trimethoprim-sulfamethoxazole was one of the least effective antibiotics against *K. pneumonia* and *P. aeruginosa* which makes this agent an inadequate single antibiotic for UTI treatment. Similar results were obtained with nitrofurantoin and trimethoprim-sulfamethoxazole in other studies [2, 10, 13].

In conclusion, a distressingly high prevalence of resistance to the majority of commonly used antibiotics in UTIs, with a decreasing trend in their activities has been monitored in this study which probably is due to the high rate of antibiotic use in Iran as the first reason. Because of the limitations in availability of new antibiotic agents, the available antibiotics should be selected based on the knowledge of the local prevalence of bacterial organisms and antibiotic sensitivities rather than on universal guidelines.

Acknowledgments

The authors would like to express their appreciations to the chairman of Urmia Imam Hospital laboratory as well as their co-operative colleagues in the microbiology laboratory.

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