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Antimicrobial susceptibility patterns of community-acquired Gram-negative uropathogens

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Drug resistance is now a public health crisis and global problem. This study was performed to identify the antibiotic susceptibility of urinary tract infections (UTI) in Sanqar City of Kermanshah, Iran. Urine specimens of 891 ambulatory patients over 18 years of age with clinically suspected UTI were referred from Physicians' Office (MD) to the Sanqar Hospital (Kermanshah, Iran) for urine culture from September 1, 2011 to December 31, 2012. These samples were cultivated in agar-blood and McConkey agar. For culture positive samples, antibiogram test was done by disk diffusion method in Muller-Hinton agar plate. The relevant results were reported based on Clinical and Laboratory Standards Institute (CLSI) criteria. Of the 891 urine specimens, 379 cases were urinary culture positive (42.28%). The most common uropathogens were *Escherichia coli* (78.1%) and *Klebsiella pneumoniae* (15%). High susceptibility patterns to: ciprofloxacin (95.3%), amikacin (93.9%), nalidixic acid (92.2%), gentamicin (89.2%) and nitrofurantoin (83.8%) among the *E. coli* isolates identified were observed. Ciprofloxacin and nalidixic acid are the most suitable antibiotics for the empirical treatment used for ambulatory patients over 18 years of age with urinary tract infections in the geographical area of this study.

Key words: Antibiotic resistance, uropathogens, antibiogram, antimicrobial susceptibility.

INTRODUCTION

Uncomplicated urinary tract infections are the most common infections encountered after respiratory infections (Schito et al., 2009). These infections if not treated lead into the increase of mortality rate, disability, hospitalization and economical costs (van et al., 2010). Unsuitable treatment can be due to the resistance of uropathogens to antibiotics. Today, drug resistance is a public health crisis and a global problem (Xiao et al., 2011).

Drug resistance model of uropathogens is different in various regions (Schito et al., 2009; Xiao et al., 2011; Gupta et al, 2001; Alós et al., 2005; Khameneh et al., 2009; Foxman, 2010; Kashef et al., 2010; Baue et al., 1996). Thus, epidemiological study of bacterial resistance is an important instrument to control the development of bacteria resistance. There was a logical association between experimental prescription (without urine culture) of the antibiotics and increase in the bacterial resistance (Gupta et al., 2001).

For proper use of antibiotics in treating urinary tract infections in each region, it is required to have a detailed knowledge of uropathogens and their drug sensitivity. This study was performed to find the antibiotic susceptibility pattern in urinary tract infections in Sanqar City located in the north east of Kermanshah, Iran.

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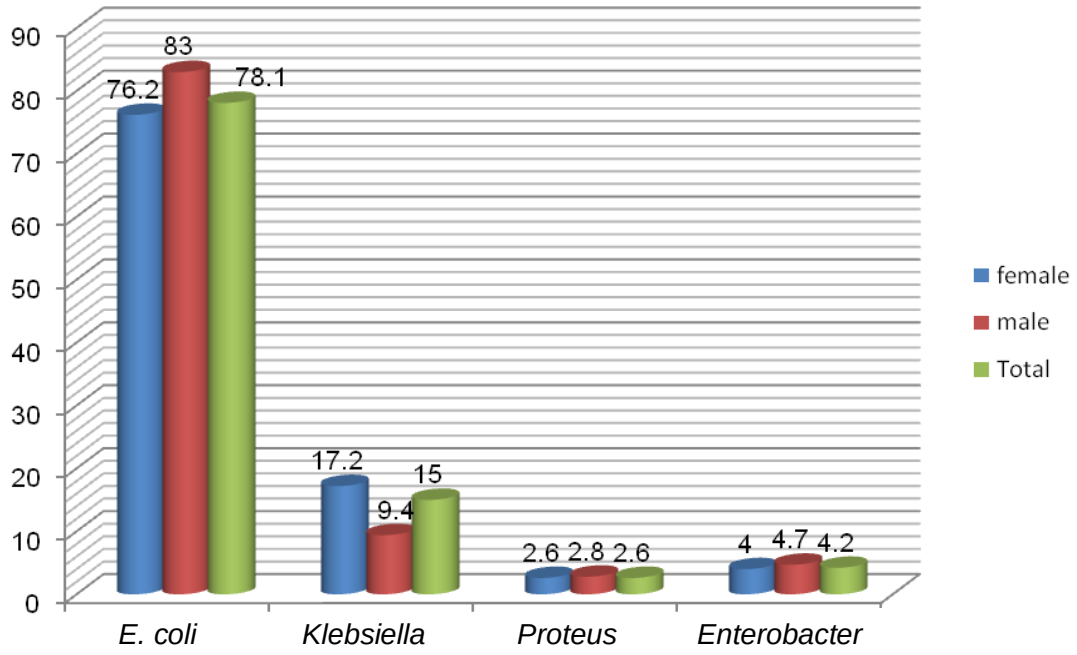

Figure 1. Frequency of isolated bacteria from positive urine samples according to patient gender.

MATERIALS AND METHODS

The Sanqar City with about 998,985 people is located in the northeastern of Kermanshah province, Iran. Imam Khomeini hospital is the only hospital in this city and is one of the subsets of Kermanshah University Medical Sciences. This observational study was carried out on 891 subjects which included 534 females and 285 males. The subjects were ambulatory patients, over 18 years of age with clinically suspected urinary tract infection, referred from Physicians' Office (MD) to Imam Khomeini hospital's laboratory of Sanqar City for urine culture from September 1, 2011 to December 31, 2012. Patients who received antimicrobials within the previous two weeks, who were suffering from renal disorders, diabetes mellitus, persons infected with HIV, on corticosteroid therapy and pregnant women were excluded; In these cases, the patient were asked to take a urine sample.

Urine specimens were collected by the mid-stream urine method in sterile dishes in the hospital laboratory. These samples were cultured in blood agar and McConkey agar for 24 h at 37°C. Then, the colonies in the medium were counted and the colonies with more than 10^5 CFU/ml of a single uropathogen was considered as culture positive. A confirmation test (TSI, triple sugar iron agar) was performed for identifying the organism.

In culture positive samples, to determine the sensitivity and resistant antibiotic of isolated specimens, sensitivity test was performed by Kirby-Bauer method in Muller-Hinton agar (Bauer et al., 1996). After incubation and determination of zone of inhibition, we determined the growth rate of the sensitive and resistant microorganisms. The applied antibiotic discs were made by Iran medicine Antibody Company.

The applied discs for bacteria isolated from culture media included antibiotics commonly used for treatment of uncomplicated UTI: cephalexin (CF, 30 µg), Ciprofloxacin (CP, 5 µg), Ampicillin (AM, 5 µg), Nitrofurantoin (NI, 50 µg), Gentamicin (GM, 10 µg), Trimethoprim-Sulfamethoxazole (SXT, 30 µg), nalidi-xic acid (NA, 30 µg), Amikacin (AK, 30 µg). After incubation for 24 h, Muller-Hinton agar was investigated and its results were reported based on the National Committee on Clinical Laboratory Standards (NCCLS) criteria (CLSI, 2012). The collected data was statistically analyzed using the SPSS 18 software. The findings were reported in number of isolates tested against each antimicrobial agent and percentage of isolates susceptible to antimicrobial agent.

RESULTS

Over a 12-month period (September 1, 2011 to December 31, 2012), 819 urine samples from Ambulatory patients over 18 years of age with clinically suspected UTI were analyzed, of which 65.2% (534/819) were females and 34.8% (285/819) males. The overall prevalence of positive urine culture was 46.28% (379/819). It was 51.12% (273/534) for females and 37.19% (106/253) for male's subjects.

Most urinary pathogens isolated were *Escherichia coli* 78.1% (296/379), *Klebsiella pneumoniae* 15.04% (57/379) for men and women. Distribution of pathogens isolated from culture samples showed no statistically significant difference in both sexes (Fisher's Exact Test = 3.861, P = N.S) (Figure 1).

Analysis according to patient age showed that the highest prevalence of positive urine culture was in the age group 25 to 34 years (158 cases, 41.69%) followed by the age group 18 to 24 years (78 cases, 20.58%). *E. coli* infections were more prevalent in the age group 25 to 34 years (129 cases, 81.65%), *K. pneumoniae* in the age group 34 to 44 years (11case, 16.92%), *Proteus mirabilis* in the age group 19 to 24 years (3 cases, 3.85%) and *Enterobacter aerogenes* in the age group 45 to 60 years

Table 1. Frequency of isolated bacteria from positive urine samples according to patient age.

Age (years)	Organism				
	E. coli	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Enterobacter aerogenes*	Total (%)
19-24 n (%)	59 (75.64)	12 (15.38)	3 (3.85)	4 (5.13)	78 (20.58)
25-34 n (%)	129 (81.65)	19 (12.03)	5 (3.16)	5 (3.16)	158 (41.69)
35-44 n (%)	50 (76.92)	11 (16.92)	1 (1.54)	3 (4.62)	65 (17.15)
45-60 n (%)	40 (75.47)	9 (16.98)	1 (1.89)	3 (5.67)	53 (13.98)
>60 n (%)	18 (72)	6 (24)	0 (0)	1 (4)	25 (6.6)
Total n (%)	296 (78.10)	57 (15.04)	10 (2.64)	16 (4.22)	379 (100)

Table 2. Resistance frequency of Gram-negative bacteria isolated from positive urine samples to commonly used antibiotics.

Bacteria	*E. coli* (n = 296)		*Klebsiella pneumoniae* (n = 57)		*Proteus mirabilis* (n = 10)		*Enterobacter aerogenes* (n = 16)	
	Isolated[1]	R[2] (%)	Isolated	R (%)	Isolated	%	Isolated	R (%)
Cephalexin	138	46.62	29	50.88	4	40	5	-
Ciprofloxacin	14	4.73	2	3.51	0	0	0	0
Ampicillin	251	84.8	55	96.49	9	90	15	-
Nitrofurantoin	48	16.22	16	28.07	3	30	7	43.75
Gentamicin	32	10.81	12	21.05	0	0	5	31.25
Co- Trimoxazol	115	38.85	21	36.84	3	30	5	31.25
Nalidixic acid	23	7.77	6	10.53	0	0	0	0
Amikacin	18	6.08	3	5.26	1	10	0	0

[1]Number of isolates tested against each antimicrobial agent. [2]Percentage of isolates resistance to antimicrobial agent.

(3 cases, 5.67%) (Table 1).

The highest antibiotic resistance of *E. coli* strains was reported to ampicillin (84.8%), cephalexin (46.62%) and trimethoprim-sulfamethoxazole (38.85%). Among *K. pneumoniae* strains isolated, the highest drug resistance was found for ampicillin (96.49%), cephalexin (50.88%), trimethoprim- sulfamethoxazole (36.84%) and nitrofurantoin (28.07%).

P. mirabilis strains showed the highest resistance to Ampicillin (90%), Cephalexin (40%), trimethoprim-sulfamethoxazole and nitrofurantoin (30%), *E. aerogenes* showed the highest resistance to nitrofurantoin (43.75%) and gentamicin (31.25%) (Table 2).

DISCUSSION

In this study, of the 819 urine samples from ambulatory patients over 18 years of age with clinically suspected UTI, 379 (46.28%) had positive urine culture. The results of our study show that the overall prevalence of positive urine culture UTI was 46.28%. It was higher in female subjects (51.12%) than in males (37.19%).

The reason for males being less prone to UTIs may be attributed to their longer urethra. These results are in agreement with other studies carried out around the world (Akram et al., 2007; Shaifali et al., 2012; Jeremy et al., 2011).

Our study shows that highest prevalence of positive urine culture was in the age group 25 to 34 years (41.69%) followed by the age group of 18 to 24 years (20.58%). In other words, more cases of UTIs were recorded among young and middle age patients (20-49 years, 51.04%) which are similar to trends reported in other studies (Akram et al., 2007; Alós et al., 2005; Jeremy et al., 2011). This may be because sexual activity is more common among these age groups.

In our investigation, the most common pathogens isolated from positive urine cultures were *E. coli* strains (78.1%) and *K. pneumoniae* (15.04%). Other studies carried out in various regions have also shown that the most common uropathogens were *E. coli* and *K. pneumoniae*. It can be due to the fact that Gram negative bacteria in are abundantly present in urinary tract system. The prevalence percentages of pathogens isolated in different regions are in agreement with our study (Kurtaran et al., 2010; Farajnia et al., 2009; Mashouf et al., 2009; Moinzadeh et al., 2013). Several studies have reported lesser or a higher prevalence (Bours et al, 2010; Ranjbar et al, 2009), because of the different quality and

quantity of treatment of urinary infections and society health level in various regions.

In this study, *E. coli* strains showed the highest antibiotic resistance to Ampicillin (84.8%), Cephalexin (46.62%) and Trimethoprim-Sulfamethoxazole (38.85%). In most of the studies performed in Iran and other countries (despite the percentage different), the highest antibiotic resistance of *E. coli* were found for ampicillin, trimethoprim- sulfamethoxazole and nalidixic acid. In other studies after Ampicillin, the highest resistance was for cephalexin and co-trimoxazol (McLoughlin and Joseph, 2003; Yüksel et al., 2006; Grude et al., 2005; Farrell et al., 2003; Moinzadeh et al., 2013; Kashef et al., 2010). Although, these results differ from some published studies from Iran, they are consistent with those of the highest antibiotic resistance of *E. coli* which were found to be Trimethoprim-Sulfamethoxazole 90% (Pourakbari et al., 2012), 66% (Valavi et al., 2013) and Nalidixic acid 59.7% (Ayatollahi et al., 2013).

In our study, *E. coli*, *K. pneumoniae* and *P. mirabilis* strains had the highest percentages of resistance to ampicillin (84.8-96.49%) and cephalexin (40-50.88%), followed by trimethoprim- sulfamethoxazole (30-38.85%); this is similar to the result reported by Bours et al. (2010). While, other similar studies conducted in iran have shown a high resistance to ampicillin and trimethoprim-sulfamethoxazole, respectively (Mashouf et al., 2009; Farrell et al., 2003; Bauer et al., 1996; Khameneh et al., 2009; Moinzadeh et al., 2013). This difference could be due to improper use of antibiotics by patients or physicians (according to the drug culture in Iran), and also because of the increased use of antibiotics in livestock breeding.

E. aerogenes strains had the highest resistance to ampicillin (93.75%) and Nitrofurantoin (43.75%), followed by gentamicin and trimethoprim- sulfamethoxazole (31.25%). These results are similar to that of Moinzadeh et al. (2013) and Kashef et al. (2010) but differ from Moinzadeh et al. (2013) and Valavi et al. (2013) that show nalidixic acid (100%) and ceftriaxon (71.4%). Resistance of *K. pneumoniae* (96.49%) to ampicillin is nearly similar to the results of the study performed in Arabia during 1999 to 2002 (Kader et al., 2004). Limitation of our study is, not considering ESBL.

Conclusion

In this study, Ciprofloxacin and Nalidixic acid were the most suitable antibiotics for the empirical treatment for ambulatory patients with urinary tract infections in the study's geography region (Sanqar City, Kermanshah, Iran).

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