British Journal of Medicine & Medical Research
1(2): 45-56, 2011

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Frequency and Antibiotic Susceptibility Pattern of Uro-Pathogens Isolated from Community and Hospital-Acquired Infections in Saudi Arabia – A Prospective Case Study

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Research Article

Received 6th February 2011
Accepted 2nd April 2011
Online Ready 16th April 2011

ABSTRACT

Objective: Urinary tract infection (UTI) remains a worldwide therapeutic problem, not only as a nosocomial disease but also as a community-acquired infection. Antibiotic resistance of urinary tract pathogens has increased worldwide. Infectious Diseases Society of America (IDSA) recently recommended that each hospital should determine the locally establish mechanisms to resistance rates among uropathogens. The aim of this study was to determine the frequency of the bacterial agents that cause urinary tract infection both in outpatients as well as in hospitalized patients and to assess the antimicrobial sensitivity pattern against various types of antimicrobial agents used for treating urinary tract infections.

Materials and Methods: We carried out a prospective study of urine culture and antibiotic sensitivity testing from symptomatic outpatients and inpatients.

Results: A total 150 cases were positive for significant bacteria, out of which 83 (55.4%) were from out patients and 67 (44.6%) were from hospitalized patients. The majority of the bacteria were isolated from female (78.7%) while the remaining was from male (21.3%). In the present study *Escherichia coli* was the predominant pathogen for UTI in both the groups. *Klebsiella sps* was the second common organism in hospital acquired infection followed by *Pseudomonas. sps*. Analysis of the frequency of bacterial isolates according to the age of the patients revealed that *Escherichia coli and Klebsiella* infections were more prevalent in the age groups (>20-49 years) and *Pseudomonas* infections were more prevalent in children and the elderly (<20 years and

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>50 years, respectively) and *Salmonella typhi* were isolated from elderly (>60 years age) patient from community–acquired infection. The mean susceptibility was high for Imipenem (98.8%), Amikacin (53.2%), Gentamicin (52.3%) and Ciprofloxacin (50.5%) but low for Ampicillin (34.2%), Norfloxacin (40.4%), Nitrofurantoin (44.5%), and Co-trimoxazole (46.7%). High prevalence of Multi-drug resistance (MDR) isolates were recovered (53%) which showed 3 or more antibiotics resistance.
Conclusion: Current knowledge of the antibiotic resistance patterns of uro-pathogens in specific geographical locations is an important factor for choosing an appropriate empirical antimicrobial treatment rather than on universal guidelines.

Keywords: Frequency; urinary tract infection; *antibiotic susceptibility; Saudi Arabia;*

1. INTRODUCTION

Urinary tract infections (UTI) are one of the most common infectious diseases, and nearly 10% of people will experience a UTI during their lifetime (Hoberman et al., 1997; Delanghe et al., 2000). UTI are the most common infections after upper respiratory tract infections (Hryniewicz et al., 2001). The infections may be symptomatic or asymptomatic, and either type of infection can result in serious sequelae if left untreated (Pezzlo, 1988). It often results in serious complications like secondary bacteremia and sepsis leading to a rise in the hospital costs and mortality. Although several different microorganisms can cause UTI, including fungi and viruses, bacteria are the major causative organisms and are responsible for more than 95% of UTI cases (Bonadio et al., 2001). *Escherichia coli* are the most prevalent causative organism of UTI and are solely responsible for more than 80% of these infections.

In recent years, bacterial resistance to different antibiotics has raised dramatically leaving physicians with few therapeutic options*. Methicillin resistant Staphylococcus aureus* (MRSA), extended-spectrum β-lactamase (ESBL) producing organisms and *vancomycin resistant enterococci (VRE)* have become common hospital problems. Since these rates of resistance to antibiotics differ from region to region, in making an appropriate choice of empiric or definitive therapy for UTI, it is useful to avail of information on prevailing levels of antimicrobial resistance among common urinary pathogens.

An accurate and prompt diagnosis of UTI is important in shortening the disease course and for preventing the ascent of the infection to the upper urinary tract and renal failure. Treatment of UTI cases is often started empirically. Therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens. However, because of the evolving and continuing antibiotic resistance phenomenon, regular monitoring of resistance patterns is necessary to improve guidelines for empirical antibiotic therapy.

2. MATERIAL AND METHODS

2.1 DESIGN OF THE STUDY

This study was conducted and samples were obtained from patients admitted to general hospitals in Qassim province at Saudi Arabia from March to June, 2010. The urine specimens were collected from outpatients and in patients suspected of having a UTI, and transported to the bacteriology laboratory within 2 hours of collection or refrigerated for 4 hours before processing. Patient age ranged from 1.5 to 65 years. From these urine specimens of symptomatic UTI patients 150 bacterial isolates were identified by conventional methods.

2.2 COLLECTION METHOD

While collecting urine for culture, care was taken to avoid contamination with normal flora of the anterior urethra or perineal skin. The common method of collection is midstream clean catch. The majority of samples were midstream urine specimen, others included catheterized urine samples.

2.3 ISOLATION AND IDENTIFICATION OF BACTERIA

Culture was done by the calibrated loop technique delivering 0.001ml of urine and plated on Cystine -Lactose-Electrolyte-Deficient (CLED) agar plates. Each plate was inoculated with 6 tests, each in duplicates, after overnight incubation at 37 degrees. The number of colonies in the impression area was counted, and if over 25 colonies were present, the original urine sample was known to have contained greater than $>10^5$ cfu/mLorganisms per milliliter, indicating significant bacteriuria. Identification of isolates was done using standard microbiological techniques (Collee et al., 1997).

2.4 THE ANTIBIOTIC SUSCEPTIBILITY TEST

Antimicrobial susceptibility of isolates was tested by the disk diffusion method according to the National Committee on Clinical Laboratory Standards (NCCLS) recommendations, using Mueller—Hinton medium. Antimicrobial agents tested were amikacin, gentamicin, ciprofloxacin, nitrofurantoin, nalidixic acid, Co-trimoxozole, ampicillin, cephalexin, Imipenem, oxacillin, and vancomycin (Oxoid.UK). *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853 was used as quality control strains. Interpretative criteria for each antimicrobial tested were those recommended by the Clinical and Laboratory Standards Institute (CLSI-2005).

2.5 DETECTION OF MRSA

All isolates in *Staphylococcus species* were tested for susceptibility to oxacillin by the agar screen method using 6 µg/ml oxacillin as recommended by the NCCLS (Brown, 2005). The *S. aureus* strains ATCC 25923 were used as negative and positive controls respectively. Agar plates were incubated at 35°C and read at 24 hours and 48 hours incubation. Organisms growing on the plate were considered to be methicillin resistant.

2.6 MRSA SCREENING FOR DECREASED VANCOMYCIN SUSCEPTIBILITY

Vancomycin resistance was tested by vancomycin agar screening test (Tiwari and Sen, 2006) whereby MRSA isolates were spot inoculated into Mueller- Hinton agar supplemented with 6 g/ml of vancomycin from 0.5 McFarland standard suspensions. The plates were incubated at 35°C for 24 h as recommended by the CLSI (2005). Any isolates growing two or more colonies on this agar would be considered as positive.

3. RESULTS

3.1 ISOLATION AND IDENTIFICATION OF BACTERIA

The pathogens causing urinary tract infections (UTI) are well known. *Escherichia coli* was the main etiologic agent in community as well as hospital acquired infections. A total of 150 urine bacteria were isolated and identified. The prevalence of community –acquired urinary tract infection was 55.3% (n=83) and hospital-acquired urinary tract infection was 44.7% (n=67). The majority of the bacteria were isolated from female 118 (78.7%) while the remaining 32 (21.3%) were from male. In the present study *Escherichia coli* was the predominant pathogen in both the groups. *Klebsiella sps* was the second common organism in hospital acquired infection followed by *Pseudomonas sps*. (Table 1). Analysis of the frequency of bacterial isolates according to the age of the patients revealed that *Escherichia coli and Klebsiella* infections are more prevalent in the age groups (>20-49 years) and Pseudomonas infections are more prevalent in children and the elderly (<20 years and >50 years) and *salmonella typhi* were isolated from elderly (>60 years age) patient from community –acquired infection. Age and gender distribution of the cases is shown in Tables 2 & 4.

Table 1. Frequency of urinary bacterial isolates in Community and hospital infections

Bacteria	Total isolate	Inpatients No. (%)	Outpatients No. & (%)
Escherichia coli	69	40 (48.1)	29 (43.2)
Klebsiella pneumoniae	30	16 (19.2)	14 (20.8)
Pseudomonas aeruginosa	12	6 (7.2)	6 (8.9)
Enterococcus species	27	13 (15.6)	14 (20.8)
Staphylococcus aureus	4	3 (3.6)	1 (1.4)
Proteus mirabilis	6	3 (3.6)	3 (4.4)
Salmonella typhi	2	2 (2.4)	0 (0)
Total	**150**	**83 (55.4)**	**67 (44.6)**

Table 2. Prevalence of UTI in different age groups and genders

Age group	Female no. (%)	Male no. (%)
0-5	8 (6.8)	4 (12.5)
5-10	12 (10.1)	1 (3.1)
10-20	3 (2.5)	1 (3.1)
20-30	34 (28.8)	0 (0)
30-40	25 (21.2)	4 (12.5)
40-50	6 (5.1)	3 (9.4)
≥50	30 (25.4)	19 (59.4)
Total	**118 (78.7)**	**32 (21.3)**

The results of our study showed that among the causative organisms of UTI, Enterobacteriaceae are the predominant pathogens, followed by Gram-positive cocci. *Escherichia coli* predominated across the two groups (community- acquired =48.1%, and hospital- acquired =43.2%) followed by *Klebsiella species* (community–acquired =19.2%, and hospital acquired =20.8*%), Enterococcus faecalis* (community–acquired=10.8%, hospital-acquired =19.4%), *Pseudomonas aeruginosa* (community–acquired=7.2%, hospital-acquired =8.9%), *Proteus mirabilis* (community- acquired =3.6%, and hospital- acquired 4.4%) and *Staphylococcus aureus* (community-acquired =3.6%, hospital- acquired =1.4%). These findings are consistent with reports published from other countries (Vromen, 1999; Dromigny et al., 2002; Kahlmeter, 2003). The least value was obtained for *salmonella typhi* (community–acquired=2.4%, and hospital-acquired =0.0%) (Table 1).

3.2 ANTIMICROBIAL SUSCEPTIBILITIES OF GRAM NEGATIVE BACILLI

Antibiotic susceptibility pattern of uro-pathogens (Gram negative Bacilli) are as follows (Table 3):

Escherichia coli: Imipinem (100%), nitrofurantoin (78%), Co-trimoxazole (70%), gentamicin (64%), amikacin (59%), tetracycline (58%), and Norfloxacin (51%). Were shows high susceptibility pattern when comparing to ciprofloxacin (30%), and ampicillin (24.6%).

Klebsiella pnemoniae: Imipinem (92%), Co-trimoxazole (79%), gentamicin (70%), and tetracycline (63%), Were shows high susceptibility pattern when comparing to ampicillin (10%). nitrofurantoin (25%) Norfloxacin (43%), amikacin (46%), and ciprofloxacin (45%).

Proteus mirabilis: Imipinem (100%), and ciprofloxacin (45%) showed high susceptibility pattern when compared to gentamicin (15%), nitrofurantoin (19%) Co-trimoxazole (20%), ampicillin (30%), Norfloxacin (33%) and amikacin (33%).

Pseudomonas aeruginosa: Imipinem (100%), ciprofloxacin (66%), gentamicin (63%), Co-trimoxazole (60%), ampicillin (59%) and amikacin (50%), showed high susceptibility when compared with tetracycline (8.3%). The lowest resistance was observed for Pseudomonas species as compared to other gram negative bacteria.

Table 3. Frequency of antimicrobial susceptibility pattern to uro-pathogens

Bacterial Isolates	No (%) n=150	Antibiotic susceptibility (%)									
		Amp	**Tet**	**Gm**	**Amk**	**Cfx**	**Nf**	**Coz**	**Nt**	**Imp**	**Ox**
Escherichia coli	69 (46%)	24.6	58	64	59	30	51	70	78	100	ND
Klebsiella spp	30 (20%)	10	63	70	46	45	43	79	25	92	ND
Proteus mirabilis	6 (4%)	30	ND	15	33	55	33	20	19	100	ND
P. aeruginosa	12 (8%)	59	8.3	63	50	66	ND	60	ND	100	ND
Salmonella typhi	2 (1.3%)	ND	50	100	50	50	50	43	ND	100	ND
Staphylococcus aureus	4 (2.7%)	10	50	50	75	100	25	35	56	100	50
Enterococcus species	27 (18%)	72	72	4.5	60	9	-	20	ND	100	ND
Mean susceptibility		**34.2**	**50.2**	**52.3**	**53.2**	**50.7**	**40.4**	**46.7**	**44.5**	**98.8**	**50**

Amp: Ampicillin; Tet: Tetracycline; Gm: Gentamicin; Amk: Amikacin; Cfx: Ciprofloxacin; NF: Norfloxacin; Coz: Cotrimoxazole; NT: Nitrofurantoin; Ox: Oxacillin; I: Imipenem; ND=Not determined.

Table 4. Distribution of uropathogens according to age and gender

Age group	***E. coli***		***Klebsiella species***		***P.aeruginosa***		***Enterococcus faecalis***		***S. aureus***		***Proteus species***		***S. typhi***	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
NB - 19	3	17	0	1	0	0	2	0	0	1	0	1	0	0
20 - 49	1	30	1	17	1	8	1	9	0	2	0	1	0	0
50 - 80	4	14	4	6	5	3	5	6	1	0	1	3	2	0
Total (150)	**8**	**61**	**5**	**24**	**6**	11	**8**	**15**	**1**	**3**	**1**	**5**	**2**	**0**

Table 5. Multi drug resistance (MDR) pattern to uro-pathogens

Bacterial Isolates	No. n=150	Resistance to Antibiotic						
		MDR Strains						
		0 Drug	1 Drug	2 Drug	3 Drug	>3 Drug	Total	%
Escherichia coli	69	8	12	12	15	22	37	53.6
Klebsiella spp	30	4	6	5	8	7	15	50
Proteus mirabilis	6	2	1	1	2	-	2	33.3
P. aeruginosa	12	1	1	3	3	4	7	58.3
Salmonella typhi	2	-	-	1	1	-	1	50
Staphylococcus aureus	4	-	-	2	1	1	2	50
Enterococcus species	27	2	4	6	7	8	15	55.5

Salmonella typhi: Imipinem (100%), gentamicin (100%), ciprofloxacin (50%), amikacin (50%) showed high susceptibility pattern when compared to Co-trimoxazole (43%). Overall, the gram negative bacilli (except Pseudomonas) were most susceptible to imipenem and nitrofurantoin.

3.3 ANTIMICROBIAL SUSCEPTIBILITIES OF GRAM POSITIVE ORGANISMS

Antibiotic susceptibility pattern of uro-pathogens (Gram positive Bacilli) are as follows (Table 3):

Staphylococcus aureus: Imipinem (100%), ciprofloxacin (100%). amikacin (75%), oxicillin (50%), gentamicin (50%), and tetracycline (50%), showed high susceptibility pattern when compared to ampicillin (10%). Norfloxacin (25%) and Co-trimoxazole (35%).

Enterococcus species: Imipinem (100%), ampicillin (10%), tetracycline (72%) and amikacin (60%) showed high susceptibility pattern when compared to Gentamicin (4.5%), ciprofloxacin (9%) and Co-trimoxazole (20%). All the *Staphylococcus* sps and *Enterococcus* isolates were sensitive to vancomycin. The antimicrobial potency and spectrum for selected antimicrobial agents of different classes against the most frequent UTI pathogens are summarized in Table 3.

The total mean antibiotic susceptibility pattern was as follows: Imipinem (98.8%), amikacin (53.2%), gentamicin (52.3%), ciprofloxacin (50.7%).Co-trimoxazole (79%), and tetracycline (50.2%), Were shows high susceptibility pattern when comparing to ampicillin (34.2%). Norfloxacin (40.4%), nitrofurantoin (44.5%) and oxicillin (50%) (Table 3).

Multi-durg resistance (MDR) pattern of uro-pathogens was as follows: *Escherichia coli*, (53.6%), *Pseudomonas aeruginosa* (58.3%), *Enterococcus sps* (55.5%), *Klebsiella sps* (50%), *salmonella typhi* (50%), *staphylococcus aureus* (50%), and *Proteus mirabilis* (33.3%) (Table 5).

4. DISCUSSION

Urinary tract infection (UTI) remains a worldwide therapeutic problem, not only as a nosocomial disease but also as a community-acquired infection (Bacheller et al., 1997; Hoberman et al., 1997; Orrett et al., 1999; Gupta et al, 2001). Early diagnosis and prompt antimicrobial treatment are required to minimize these complications (CQISUTI-1999). *Escherichia coli* (48.1. and 43.2%) are the commonest cause of UTI in our study with both community and nosocomial settings followed by *Klebsiella species* (19.2% and 20.8%), *Enterobacter species* (15.6% and 20.8) and *Pseudomonas* species (7.2% and 8.9%) respectively. These findings are similar to studies from the national survey of nosocomial UTI in the United States. The study found *Escherichia coli*, *Pseudomonas and Klebsiella species* among the top 5 pathogens (Shariti et al., 1996). The majority of the bacteria were isolated from female 118 (78.7%), while the remaining 32 (21.3%) were from male. *Escherichia coli* and *Klebsiella* infections are more prevalent in the age groups (>20-49 years) and Pseudomonas infections are more prevalent in children and the elderly (<20 years and >50 years). The frequency of UTI is greater in women as compared to men and our results were similar to these reports (Schaeffer et al., 2001). This might be owing to anatomic and physical factors (Kumar et al., 2006; Khan et al., 2004).

In our study *Escherichia coli* and *Klebsiella Sps*, isolates were found to be resistant to ampicillin (75.4% and 90% respectively) while for ampicillin, *Klebsiella Sps* was more resistant than E. coli in this region. Our results showed slightly higher resistance rate when compared to other studies done worldwide like in USA (39.1% and 8.6 % respectively) (Vromen et al., 1999) and Europe (29.8% and 14.1% respectively) (Kahlmeter, 2003). On the other hand, the rate of resistance against these antibiotics in countries like Senegal (77% and 55%), Spain, (65% and 33%), Taiwan (80% and 56%), and Israel (66% and 26%) is comparable with our results (Dromigny, 2002; Daza et al., 2001; Lau, 2004).

Escherichia coli isolates are susceptible to (78%) nitrofurantoin. However, *Klebsiella Sps (25%)* and *Proteus mirabilis* (19%) are less susceptible to nitrofurantoin; similar findings were reported in several other countries (Al-Sweih et al., 2005; Zhanel et al., 2000; Farrell et al., 2003). Whereas, this drug exhibited low resistance rate in the major part of the world (0–5.4%), despite of it's being used for many years (Honderlick et al., 2006). In the present study overall imipenem resistance was 8% for *Klebsiella pneumoniae,* whereas, other isolates of uropathogens were found to be highly sensitive to imipenem (100%). So our results are comparable with other reports (Akram et al., 2007). It is highly stable against β-lactamase and has an unusual property of causing a post antibiotic effect on gram-negative bacteria (Neu, 1992), whereas Nitrofurantoin and Flouroquinolones, Aminoglycoside and showed sensitivity of 70-90% in both groups of UTI.

The total mean antibiotic susceptibility pattern i.e., Imipinem (98.8%), amikacin (53.2%), gentamicin (52.3%), ciprofloxacin (50.7%).Co-trimoxazole (79%), and tetracycline (50.2%), was found to be high when compared to ampicillin (34.2%), Norfloxacin (40.4%), nitrofurantoin (44.5%) and oxicillin (50%). Similar reports were published in different parts of the world. Resistance prevalence is relatively lower in the more developed countries such as in North America and Europe (3 to 19%) than in developing countries such as Chile (44%), Thailand (40%) Ethiopia (55%) India (45%) and Saudi Arabia (60%) (Murray, 1991; Mouton, 1990; Dornbusch, 1990; Aseffa, 1996).

In our study, *Enterococcus faecalis* (55.5%) was found to be the most common Multi Drug Resistance (MDR) uro-pathogens followed by *Escherichia coli (*53.6%) and *Pseudomonas aeruginosa* (58.3%). MDR was defined as resistance to 3 or >3 classes of the antimicrobials (≥3drugs). Similar data is presented in other reports. On the basis of reports by antimicrobial surveillance program, isolates from Canada, USA and Latin American countries show the lowest susceptibility rates to most antimicrobial agents followed by Asian-pacific isolates and European strains (Mathai, et al., 2001; Kahlmeter, 2003; Akram et al., 2007; Kiffer et al., 2007).

In our study the most active antibiotic against all gram negative isolates were Imipenem, amikacin, gentamicin and ciprofloxacin. In contrast to other isolates high resistance was found against ampicillin, norfloxacinn, nitrofurantoin, and co-trimoxazole. Although the prevalence of pathogens in different parts of the world is somewhat similar, antimicrobial resistance patterns reported from different regions are significantly different and antimicrobial resistance increases.

5. CONCLUSION

Antibiotic resistance is becoming a big problem for the public health which threatens the lives of hospitalized individuals. The results of our study demonstrated the frequency of bacterial isolates causing urinary tract infections and their susceptibility pattern to commonly

used antibiotics. Moreover, this study concludes that, *Escherichia coli* were the predominant pathogen in both the groups. *Klebsiella Sps was* the second common organism in hospital acquired infection followed by *Pseudomonas sps* . The mean susceptibility was high for Imipenem, Amikacin, Gentamicin and Ciprofloxacin but low for Ampicillin, Norfloxacin, Nitrofurantoin, and Co-trimoxazole (46.7%). Therefore, regular monitoring is required to establish reliable information about resistance pattern of urinary pathogens for optimal empirical therapy of patients with UTI.

Infectious Diseases Society of America (IDSA) recently recommended that each hospital should determine the local establish mechanisms to resistance rates among uropathogens and that the standard antimicrobial regimens for empirical treatment of UTIs should be reassessed periodically in light of changing susceptibility pattern. Finally, we suggest that empirical antibiotic selection should be based on the knowledge of local prevalence of bacterial organism and sensitivities rather than on universal guidelines.

ACKNOWLEDGMENTS

The authors would like to thank the laboratory staff of the hospital for their assistance during their investigation, this work was partially supported by a grant from the Scientific Research Deanship at Qassim University, Saudi Arabia in the year 2010.

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