ORIGINAL ARTICLE

Antimicrobial Susceptibility Pattern of Bacteria Isolated from Patients with Urinary Tract Infection

Inam Ullah Khan, Irfan Ali Mirza, Aamer Ikram, Amna Afzal, Shamshad Ali, Aamir Hussain, Muhammad Fayyaz and Tahir Ghafoor

ABSTRACT

Objective: To determine the antimicrobial susceptibility pattern of bacterial pathogens in the patients of urinary tract infection reporting at a tertiary care hospital.

Study Design: Laboratory based study.

Place and Duration of Study: Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, from January to December 2012.

Methodology: A total of 440 culture positive bacterial isolates from 1110 urine samples; submitted over a period of one year were included in this study. Identification of bacterial isolates was done by standard biochemical profile of the organisms. The antimicrobial susceptibility of culture positive bacterial isolates was performed by disk diffusion method as recommended by Clinical Laboratory Standard Institute guidelines (CLSI).

Results: Out of the 440 culture positive urine samples, 152 (34.6%) were from indoor patients whereas 288 (65.4%) from outdoor patients. Gram negative bacteria accounted for 414 (94%) of the total isolates while rest of the 26 (6%) were Gram positive bacteria. The most prevalent bacterial isolate was *Escherichia (E.) coli* 270 (61.3%) followed by *Pseudomonas (P.) aeruginosa* 52 (12%) and *Klebsiella (K.) pneumoniae* 42 (9.5%). The susceptibility pattern of *E. coli* showed that 96.2% of the bacterial isolates were sensitive to imipenem, 85.1% to amikacin, 80.7% to piperacillin/tazobactam and 72.6% to nitrofurantoin. In case of *P. aeruginsosa,* 73% bacterial isolates were sensitive to tazobactam/piperacillin, 69.2% to sulbactam/cefoperazone and 65.38% to imipenem. The antibiogram of *K. pneumoniae* has revealed that 76.1% of the bacterial isolates were sensitive to imipenem and 52.3% to piperacillin/tazobactam. Nitrofurantoin and imipenem were the most effective antimicrobials amongst the *Enterococcus* spp. as 92.3% showed susceptibility to this bacterial isolate.

Conclusion: Majority of the bacterial isolates were sensitive to imipenem and piperacillin/tazobactam while susceptibility to most of the commonly used oral antibiotics was very low. Among the oral antimicrobials, nitrofurantoin showed good susceptibility against Enterobacteriaceae family and Gram positive organisms.

Key Words: *Antimicrobial susceptibility. Urinary pathogens. Urinary tract infection.*

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common human bacterial infections both in the community and hospital setting.[1-3] This disease affects people of all age groups, being more common in women.[4,5] In most of the cases there is a need to start prophylactic therapy before culture and sensitivity results are available. Area-specific monitoring studies aimed at obtaining knowledge about the type of bacteria responsible for UTIs and their resistance patterns may help the clinician to choose the right empirical treatment.

Ampicillin, trimethoprim/sulfamethoxazole, ciprofloxacin and nitrofurantoin are the most commonly used oral antibacterial drugs in the treatment of UTIs in community settings.[6] Hospital acquired UTIs are a serious threat especially for immunocompromised patients as it can cost a significant financial burden to the hospital management.[4] Members of the family Entero-bacteriaceae is a well known cause of urinary tract infections. *E. coli* is reported to be the most common bacteria with prevalen as high as 71% and resistance to commonly used antimicrobials.[7] Other Gram negative organisms like *P. aeruginosa* and Gram positive organisms like *Enterococcus* spp. are the common urinary tract bacteria frequently involved in hospital acquired infections.[8]

The indiscriminate use of antimicrobials has resulted in selective pressure on bacterial population with emergence of resistant mutants.[9] Extended Spectrum Beta-Lactamase (ESBL), Metallo Beta-Lactamase (MBL) and Amp-C mediated beta-lactamases are some of the enzymes produced by Enterobacteriaceae and other non-lactose fermenters causing UTIs.[9] In addition to cephalosporins, the uropathogens are also exhibiting

Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi.

Correspondence: Dr. Inam Ullah Khan, Registrar, Microbiology Department, Armed Forces Institute of Pathology, Rawalpindi.
E-mail: capt_inam@yahoo.com

Received: March 19, 2013; Accepted: July 07, 2014.

increasing resistance to antibiotics like cotrimoxazole, quinolones and nitrofurantoin. The wide array of resistance mechanism has jeopardised the empirical use of quinolones and cephalosporins. The therapeutic options to treat UTIs caused by multidrug resistant bacteria have forced the clinicians to resort to carbapenems, colistin and fosfomycin.[10,11]

The aim of this study was to determine the types of bacteria isolated from the urinary tract infections and their susceptibility pattern from patients reporting in tertiary care urology centre in order to rationalize the use of antibiotics.

METHODOLOGY

This laboratory based study was carried out in the Microbiology Department of Armed Forces Institute of Pathology, Rawalpindi. Permission was taken from institutional ethical and research committee for research purpose. Non-probability consecutive sampling was done. All culture positive urinary specimens from patients reporting at tertiary care urology centre from January to December 2012 were included in this study. Repeated samples from the same patient as well as non-culture positive were excluded.

Bacterial concentration of 10^5 cfu/ml was considered as significant attained after inoculating 0.2 ul of urine on Cysteine Lactose Electrolyte deficient agar (Oxoid, UK) using semi-quantitative strip method (MAST Bacteruritest).[12] The culture media was then incubated at 37°C for 24 to 48 hours.[12] Identification of the microorganisms was done through Gram staining, biochemical tests and serology. Analytical profile index API-20E (Biomerieux, France) was used to identify Enterobacteriaceae family and associated organisms according to manufacturer's directions.

Antibacterial susceptibility of the isolates was done using Kirby-Bauer disk diffusion method following CLSI protocol. Commercially available standard antibiotic discs (Oxoid UK) were used. The zones of inhibition were measured and recorded according to the CLSI guidelines.[13] Amikacin (30 µg), imipenem (10 µg), nitrofurantoin (300 µg), gentamicin (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), amoxicillin/clavulanic acid (20/10 µg), ciprofloxacin (5 µg), trimethoprim/ sulfamethoxazole (1.25/23.75 µg), ampicillin (25 µg), vancomycin (30 µg), linezolid (30 µg), teicoplanin (30 µg), piperacillin/tazobactam (100/10 µg) were used. *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used as control strains. The material for research purpose was made available by the institute. However, there were neither conflict of interests of authors with the material provider companies nor any financial and other gains were obtained from the companies.

The data obtained was entered in Statistical Package for Social Sciences (SPSS) version 17 for statistical evaluation. Descriptive statistics was applied to calculate mean, standard deviation for age, percentages for different variables like gender and antimicrobial susceptibility pattern of bacteria isolated in patients of urinary tract infection reporting at tertiary care urology centre.

RESULTS

Out of a total 1110 urine specimens, 440 (40%) yielded bacterial growth. The isolate yielding bacterial growth 307 (69.8%) urine cultures were from female patients and rest 133 (30.2%) male patients. Out of the positive urine cultures 152 (34.5%) were from indoor patients and rest 288 (65.4%) from outdoor patients. The age of the patients presenting with UTIs ranged from 1 to 79 years with large numbers around 48 years of age. Gram negative bacteria constituted the major bulk with a total of 414 (94%) isolates. *E. coli* 270 (61.3%), *P. aeruginosa* 52 (11.8%) and *Klebsiella pneumoniae* 42 (9.5%) accounted for the major bulk i.e. 82.7% of the total culture positive Gram negative isolates while Gram positive bacteria accounted for only 26 (5.9%) of total isolates as shown in Figure 1.

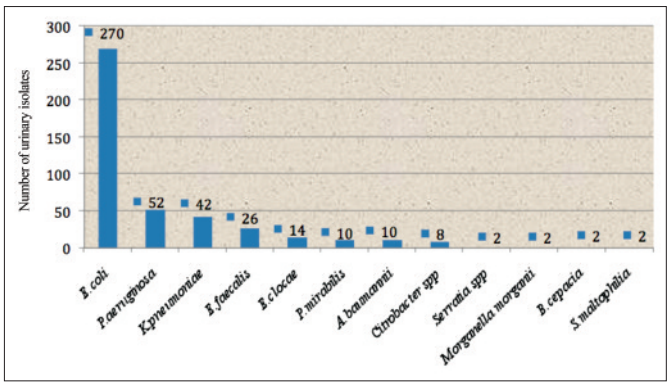

Figure 1: Spectrum of pathogens isolated (n = 440).

Table I: Antimicrobial susceptibility pattern of Enterobacteriaceae.

Antibiotics	*E. coli* n=270	*K. pneumoniae* n=42	*E. cloacae* n=14	*P. mirabilis* n=10
Ampicillin	6 (2.2%)	0	0	2 (20%)
Trimethoprim/ Sulphamethoxazole	70 (25.9%)	8 (19%)	4 (28.6%)	0
Gentamicin	132 (48.8%)	18 (42.8)	6 (42.8%)	4 (40%)
Amikacin	230 (85.1%)	20 (47.6%)	0	4 (40%)
Ciprofloxacin	58 (21.5%)	6 (14.2%)	4 (28.6%)	0
Nitrofurantoin	196 (72.6%)	2 (4.7%)	6 (42.8%)	0
Amoxicillin/ Clavulanic acid	64 (23.7%)	12 (28.6%)	0	6 (60%)
Ceftriaxone	126 (46.6%)	14 (33.3%)	8 (57.1%)	2 (20%)
Imipenem	260 (96.2%)	32 (76.1%)	14 (100%)	8 (80%)
Piperacillin/ Tazobactam	218 (80.7%)	22 (52.3%)	12 (85.7%)	10 (100%)

Susceptibility pattern of *E. coli* revealed that 260 (96.2%) of isolates were sensitive to imipenem, 218 (80.7%) to piperacillin/tazobactam, 230 (85.1%) to amikacin and 196 (72.6%) to nitrofurantoin. The susceptibility pattern of Enterobacteriaceae against various antimicrobials is shown in Table I.

The antimicrobial susceptibility pattern of *P. aeruginosa, Acinetobacter baumannii* and Gram positive *Enterococcus* spp is shown in Table II and III.

Table II: Antimicrobial susceptibility pattern of *Pseudomonas* and *Acinetobacter* spp.

Antibiotics	*P. aeruginosa* n=52	*A. baumannii* n=10
Trimethoprim/Sulphamethoxazole	-	6 (60%)
Gentamicin	4 (7.7%)	-
Amikacin	14 (26.9%)	2 (20%)
Ciprofloxacin	8 (15.4%)	4 (40%)
Nitrofurantoin	-	-
Imipenem	34 (65.3%)	2 (20%)
Piperacillin/Tazobactam	38 (73%)	8 (80%)
Ampicillin/Sulbactam	22 (42.3%)	6 (60%)
Cefoperazone/Sulbactam	36 (69.2%)	4 (40%)
Ceftazidime	30 (57.7%)	-
Cefipime	16 (30.8%)	-
Aztreonam	20 (38.5%)	-

Table III: Antimicrobial susceptibility pattern of *Enterococcus* spp. (n= 26).

Antibiotics	*Enterococcus* spp. n=26
Ampicillin	14 (53.8%)
Ciprofloxacin	8 (30.7%)
Nitrofurantoin	24 (92.3%)
Amoxicillin/Clavulanic acid	18 (69.2%)
Imipenem	24 (92.3%)
Vancomycin	26 (100%)
Tiecoplanin	26 (100%)
Linezolid	26 (100%)
Tetracycline	8 (30.7%)

DISCUSSION

Bacterial infections of the urinary tract are one of the frequent cause for seeking medical attention in community.[14] Effective management of patients suffering from bacterial UTIs commonly relies on the identification of the bacterial isolate and the selection of an effective antibiotic agent used for the treatment of bacterial organisms in question.[15] Antimicrobial resistance is a serious public health threat. Treatment failure is caused by resistance developed by different bacterial pathogens against commonly used antimicrobials.

In this study, the bulk of the urinary isolates were from female patients (70%) as UTIs are frequent in females due to short urethra. Mean age of the patients was around 48 years. The most common urinary tract bacteria was found to be *E. coli*, a frequent causative agent of UTIs. A similar study conducted at Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi in 2010 and at Mayo Hospital, Lahore in 2013 revealed *E. coli* as the most common bacteria accounting for 63% and 80% of the total culture positive isolates.[16,17] A similar study conducted in Peshawar, Khyber Pakhtunkhwa Pakistan has revealed similar results showing *E. coli* (77%) as the predominant uropathogen.[18] However, the frequency of *E. coli* as the causative agent of UTIs was found to be 80 - 90 % in two similar studies carried out in Canada and Ethiopia in the recent years.[19,20]

In this study, 96% of *E. coli* isolates were susceptible to imipenem, the result being consistent with similar study carried out at AFIP, two years ago and the study conducted in Peshawar.[16,18] These results are also similar to earlier studies carried out in India where 96% of *E. coli* isolates were susceptible to imipenem.[11] The results of this study are contrary to a similar study conducted recently in Lahore, Pakistan, where *E. coli* showed 44% resistance to carbapenems.[17] This proves the injudicious use of antimicrobials limiting the use of oral antibiotics for UTIs.

As far as the antimicrobial sensitivity of quinolones to *E. coli* is concerned, the susceptibility to ciprofloxacin was 21.5% comparable to a study conducted in Pakistan and India.[11,17,18] However, the situation is quite different with *E. coli* isolated in a study carried out in London where 94% of bacterial isolates were susceptible to ciprofloxacin.[10] These contrasting results clearly suggest the injudicious use of quinolones in this part of the world has led to deteriorating susceptibility to this important antimicrobial group.

Similarly, another important oral antimicrobial used for empirical treatment of uncomplicated UTIs in our setup is trimethoprim/sulfamethoxazole. The susceptibility of *E. coli* to cotrimoxazole was found to be only 26% in this study and 46% in another local study, which is quite low as compared to similar studies conducted in Tunisia and other parts of the world.[8,18]

In this study, the susceptibility of *E. coli* to nitrofurantoin was 72.6% compared to 94% in a study done in London.[10] Nitrofurantoin is effective against many Gram positive and Gram negative urinary isolates and activity of this antimicrobial is greatly enhanced at pH 5.5 and below. It is a cheap antimicrobial and can be given orally for months for the suppression of chronic UTIs. All of our enterococcal isolates were sensitive to nitrofurantoin. It shows that nitrofurantoin is still effective against majority of the urinary isolates and can be used prophylactically for recurrent urinary tract infections.

The second most common urinary isolate in this study was *Pseudomonas aeruginosa* which is contrary to other contemporary studies where the second commonest reported isolates were *Staphylococcus* spp. and

K. pneumoniae.[17,18,21] Carbapenems, amikacin, ceftazidime and antipseudomonal penicillin such as piperacillin are the recommended antibiotics to treat UTIs caused by *P. aeruginosa*. The antimicrobial susceptibility profile of *Pseudomonas aeruginosa* in this study revealed that a good percentage of the isolates were sensitive to antipseudomonal penicillins followed by imipenem and ceftazidime. These results are slightly different from an earlier study carried out at the same institute (2010), when 86% of the isolates were sensitive to ceftazidime and imipenem followed by antipseudomonal penicillins (76%).[16] This is a worrying trend with an indication that *P. aeruginosa* is gradually developing resistance against carbapenems and antipseudomonal third generation cephalosporins.

In this study, *Klebsiella* spp. isolates showed better susceptibility against Imipenem followed by sulbactam/ cefoperazone and amikacin. The antimicrobial susceptibility of Enterobacteriaceae other than *E. coli* has revealed that imipenem was the most effective antibiotic similar to other local studies.[18] *In vitro* activity of oral antimicrobials such as trimethoprim/ sulphamethoxazole and ciprofloxacin was quite low comparable to other studies performed in Pakistan, which is a worrying trend as far as the oral antibiotics are concerned.[18] The results of this study were almost similar to a study carried out at the same institute in 2010.[16]

The antimicrobial susceptibility of *Acinetobacter* spp. causing UTI's in the studied population revealed high degree of resistance to almost all the routinely used antibiotics necessitating its susceptibility testing for newer drugs. All the uropathogens showed high degree of resistance to trimethoprim/sulphamethoxazole. This is possibly due to the opportunistic nature of the organism and its versatility in causing nosocomial infections in hospitalized patients especially those fitted with catheters.

The antibiogram of enterococcal isolates showed that all isolates were susceptible to vancomycin, teicoplanin and linezolid. The antimicrobial susceptibility of the enterococcal isolates against amoxycillin/clavulanic acid and ciprofloxacin was quite low. However, no vancomycin resistant enterococcus was isolated in this study.

Resistance to antimicrobials has been noted ever since the first use of these agents and is increasing with each passing day.[22] The fact that 98% of *E. coli* and 100% of *K. pneumoniae* isolates were resistant to amoxicillin/ clavulanic acid and ampicillin is of immense importance which implies that these antibiotics can no longer be considered for empirical therapy in urinary tract infection.

Empirical treatment for nosocomial UTIs with multi-drug resistant isolates remains challenging with many authorities recommending parenteral carbapenem especially where ESBL producing isolates are involved.[23] The increasing rates of resistance to uropathogenic isolates warrants evaluation of other antimicrobials such as fosfomycin which can safely be given orally and is highly effective against many uropathogens.[24] The results of this study will benefit clinicians to know the local pattern of antimicrobial susceptibilities and formulate the empirical antibiotic strategies in patients presenting with UTIs.

CONCLUSION

E. coli was the predominant pathogen causing UTIs in our population presenting at tertiary care urology centre followed by *Pseudomonas* spp. A majority of the isolates were sensitive to imipenem and piperacillin/tazobactam limiting the use of oral antimicrobials commonly used to treat UTIs. As far as the oral antibiotics are concerned, nitrofurantoin revealed encouraging results proving to be the only effective oral antibiotic in this study. As drug resistance among bacterial pathogens is an evolving process, routine surveillance and monitoring studies should be conducted in different parts of the country to provide physicians, an effective knowledge regarding the empirical treatment of UTIs in that particular area.

REFERENCES

1. Akortha EE, Ibadin OK. Incidence and antibiotic susceptibility pattern of *Staphylococcus aureus* amongst patients with urinary tract infection in UBTH Benin City, Nigeria. *Afr J Biotechnol* 2008; **7**:1637-40.
2. Dalela G, Gupta S, Jain DK, Mehta P. Antibiotic resistance pattern in uropathogens at a tertiary care hospital at Jhalawar with special reference to ESBL, Amp-C β-Lactamase and MRSA production. *J Clin Diagn Res* 2012; **6**:645-51.
3. Bhattacharya S. ESBL- From petri dish to the patient. *Indian J Med Microbiol* 2006; **24**:20-4.
4. Enayat K, Fariba F, Bahram N. Asymptomatic bacteriuria among pregnant women referred to outpatient clinics in Sanandaj, Iran. *Int Braz J Urol* 2008; **34**:699-707.
5. Rock W, Colodner R, Chazan B, Elias M, Raz R. Ten years surveillance of antimicrobial susceptibility of community acquired *Escherichia coli* and other uropathogens in Northern Israel. *Israel Med Assoc J* 2007; **9**:803-5.
6. Jancel T, Dudas V. Management of uncomplicated urinary tract infections. *West J Med* 2002; **176**:51-5.
7. Thabet L, Messadi AA, Meddeb B, Mbarek A, Turki A, Ben RS. Bacteriological profile of urinary tract infection in women in Aziza Othmana Hospital: a 495 cases study. *La Tunisie Medicale* 2010; **88**:898-901.
8. Minardi D, d'Anzeo G, Cantoro D, Conti A, Muzzonigro G. Urinary tract infections in women: etiology and treatment options. *Int J Gen Med* 2011; **4**:333-43.
9. Patel MH, Trivedi GR, Patel SM, Vegad MM. Antibiotic susceptibility pattern of urinary isolates of gram negative bacilli with special reference to Amp-C β-lactamase in a Tertiary Care Hospital. *Urol Ann* 2010; **2**:7-11.

10. Bean DC, Krahe D, Wareham DW. Antimicrobial resistance in community and nosocomial isolates of *Escherichia coli* urinary tract isolates, London 2005-2006. *Ann Clin Microbiol Antimicrob* 2008; **7**:13.

11. Eshwarappa M, Dosegowda R, Aprameya IV, Khan MW, Kumar PS, Kempegowda. Clinico-microbiological profile of urinary tract infection in south India. *Indian J Nephrol* 2011; **21**:30-36.

12. Butt T, Leghari MJ, Mahmood A. *In-vitro* activity of nitrofurantoin in *Enterococcus* urinary tract infection. *JPMA* 2004; **54**:466-9.

13. Clinical and Laboratory Standard Institute (CLSI). Performance standard for antimicrobial susceptibility testing: twenty-second informational supplement M100-S22. Wayne, PA: *CLSI;* 2012.

14. Kebira AN, Ochola P, Khamadi SA. Isolation and antimicrobial susceptibility testing of *Escherichia coli* causing urinary tract infections. *J Appl Biosci* 2009; **22**:1320-25.

15. Water G, Harrison B, Kunin G. Urinary tract infection. *N Engl J Med* 1996; 248-50.

16. Amjad A, Mirza IA, Abbasi SA, Farwa U, Sattar A, Qureshi ZA. Spectrum and antimicrobial susceptibility pattern of pathogens causing urinary tract infection: experience in a tertiary care setting. *Infect Dis J* 2011; **20**:297-301.

17. Sabir S, Anjum AA, Ijaz T, Ali MA, Khan MR, Nawaz M. Isolation and antibiotic susceptibility of *E. coli* from urinary tract infections in a tertiary care hospital. *Pak J Med Sci* 2014; **30**: 389.

18. Shahzad KA, Ullah F, Muhammad K, Khatoon F, Qazi MH, Ahmed I. Multiple drug resistance patterns in urinary tract infection patients in Peshawar, Khyber Pukhtunkhwa (KPK) Pakistan. *J Inf Mol Biol* 2013; **1**:67-70.

19. Ronald A. The etiology of urinary tract infection: traditional and emerging pathogens. *Am J Med* 2002; **113**:14-9.

20. Tessema B, Kassu A, Mulu A, Yismaw G. Predominant isolates of urinary tract pathogens and their susceptibility patterns in Gonder Univesity Teaching Hospital, Northwest Ethiopia. *Ethio Med J* 2007; **45**:61-7.

21. Chin BS, Kim MS, Han SH. Risk factors of all cause in-hospital mortality among Korean elderly bacteremic urinary tract infection (UTI) patients. *Arch Gerontol Geriatr* 2011; **52**:50-5.

22. Sefton AM. The impact of resistance on the management of urinary tract infections. *Int J Antimicrob Agents* 2000; **16**: 489-91.

23. Matsumoto T, Muratani T. Newer carbapenems for urinary tract infections. *Int J Antimicrob Agents* 2004; **24**:35-8.

24. Pullukcu H, Tasbakan M, Sipahi OR, Yamazhan T, Aydemir S, Ulusoy S. Fosfomycin in the treatment of extended spectrum β-lactamase producing *Escherichia coli* related lower urinary tract infection. *Int J Antimicrob Agents* 2007; **29**:62-5.