ORIGINAL ARTICLE

Susceptibility Pattern of Urinary Pathogens in a Tertiary Care Hospital

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Abstract

Objective
To determine the susceptibility pattern of urinary pathogens in a tertiary care hospital of Pakistan.

Study Design
Laboratory based retrospective study.

Place and Duration of Study
Department of Pathology Combined Military Hospital Sialkot, from April 2016 to March 2018.

Methodology
Urine samples were submitted in laboratory. 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 2668 urine samples submitted over a period of two years, 220 turned out to be culture positive. Out of these culture positives, 34.1% were from indoor patients and 65.9% from outdoor. Female patients constituted 151 (68.6%) urine samples, while 69 (31.4%) were from male. Male to female ratio was 1:2.2. Mean age of patients was 40 years. Gram negative bacteria accounted for 196 (89%) of the total isolates, Gram positive bacteria 17 (7.8%) while 7 (3.2%) were *Candida* spp. The most prevalent bacterial isolate was *Escherichia coli* 145 (65.9%) followed by *Klebsiella pneumoniae* 26 (11.8%), *Enterococcus* spp17 (7.8%), *Pseudomonas aeruginosa* 08 (3.6%), *Candida* spp 07 (3.2%), *Serratia* spp 05 (2.3%) while 04 (1.8%) each for *Enterobacter* spp, *Citrobacter* spp and *Proteus* spp. *E.coli* showed highest susceptibility (96.5%) to fosfomycin, 95.2% followed by nitrofurantoin (92.4%) and imepenem(86.9%). The antibiogram of *Klebsiella pneumoniae* revealed 88.5% of the bacterial isolates sensitive to fosfomycin and 84.6% to imipenem. In case of *P.aeruginsosa*, sensitivity to imipenem turned out to be87.5% followed by amikacin (75%). Vancomycin, linezolid and fosfomycin were the most effective antimicrobials amongst the *Enterococcus* spp, showing 100%, 94.1% and 94.1% susceptibility respectively.

Conclusion
Majority of the bacterial isolates of *Enterobacteriaceae* family were sensitive to fosfomycin, nitrofurantoin, imepenem and amikacin. While in case of Gram positive isolates, vancomycin and linezolid showed good susceptibility in addition to fosfomycin and nitrofurantoin.

Key Words
Urinary tract infection, Antimicrobial susceptibility, Urinary pathogens.

Introduction

Urinary tract infection (UTI) is considered as one of the most prevalent infectious disease having incidence of 18/1000 persons per year in the general population.[1,2] UTI may be diagnosed clinically as well as by diagnostic means. Microbiologically UTI is defined as the presence of a growth of more than 10^5 colony forming units (CFU) of bacteria per ml of urine for asymptomatic individual and much lower for symptomatic individuals (~10^3 CFU/ ml). Clinically, UTIs are categorized as uncomplicated or complicated. Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities. [3,4] UTIs are a significant cause of morbidity in infant boys, older men and females of all ages. Serious sequel includes frequent recurrences, pyelonephritis with sepsis, renal damage. Several risk factors are associated with lower UTIs, including a prior UTI, female gender, sexual activity, obesity, vaginal infection, diabetes and genetic susceptibility.[5,6]

Both in the community as well as in hospital settings, UTIs are one of the most common human bacterial infections.[7-9] 70–80% of complicated UTIs were reported to be attributable to indwelling catheters in the United States.[10] Most of the UTI cases are caused by bacteria that normally multiply at the opening of the urethra and move by retrograde ascent from the fecal flora through urethra to the urinary bladder and kidneys

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especially in cases of females who have a shorter and wider urethra than males. In males the organisms often originate from the sub prepucial sac, while patients with prostate problems such as inflammation and babies particularly those born with physical problems (congenital abnormalities) of the urinary system are susceptible to UTIs.[11]

A wide range of pathogens including *E.coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus* spp and *Staphylococcus saprophyticus* may cause UTIs, but *E.coli* has been reported as the most common etiologic agent.[12] According to the University of Michigan, up to 40% of women will develop UTI at least once during their lives and a significant number of these women will have recurrent UTIs.[13] Emergence of antimicrobial resistance is becoming a growing public health concern where the microorganism is able to survive exposure to antibiotic treatment.[14] Early identification of organisms causing UTIs and to treat them as soon as possible is of utmost importance in order to avoid not only long term complications but also to reduce the risk of any significant morbidity.

Effective management of UTI requires understanding of the pathogenesis of UTI along with aetiological agent and its susceptibility profile. For prompt diagnosis of UTI, bacteriological culture of urine plays an important role. The wide variety of resistance mechanisms had resulted in emergence of multidrug resistant (MDR) bacteria which forced the clinicians to switch over to carbapenems, fosfomycin and colistin. This study was aimed to make the antibiogram of UTI pathogens isolated in our settings which will not only help in rational use of antibiotics but also help to formulate effective antibiotics policy for guidance in UTI cases.

Methodology

This laboratory based study was carried out in the Pathology Department of Combined Military Hospital (CMH) Sialkot, which receives samples from patients mostly belonging to Sialkot and surrounding areas. Permission was taken from hospital ethical and research committee for research purpose. Non-probability consecutive sampling was done. All culture positive urinary specimens from patients reporting at Pathology Department of CMH Sialkot from April 2016 to March 2018 were included in this study. Repeated samples from the same patient showing growth of similar microorganism and similar sensitivity pattern were excluded. Bacterial concentration of 10^5CFU/ml was considered as significant attained after inoculation of urine on Cysteine Lactose Electrolyte deficient agar (Oxoid, UK) using semi-quantitative strip method (MAST Bacteruritest).[15] The culture media was incubated aerobically at 35°C ± 2 for 18 to 24 hours.[15] 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 with 0.5 McFarland standard was done on Prepared Mueller-Hinton agar (MAST Diagnostics, UK) by 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.[16] Amikacin (30 µg), imipenem (10 µg), nitrofurantoin (300 µg), gentamicin (30 µg), ceftriaxone (30 µg), amoxicillin/clavulanic acid (20/10 µg), doxycycline (30 µg), ciprofloxacin (5 µg), tigecycline (15 µg), trimethoprim/ sulfamethoxazole (1.25/23.75 µg), ampicillin (25 µg), vancomycin (30 µg), linezolid (30 µg), piperacillin/tazobactam (100/10 µg), cefipime (30 µg), and fosfomycin tromethamine (200 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 hospital. 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 UTIs reporting at Pathology Department of CMH Sialkot.

Results

Out of 2668 urine samples submitted over a period of two years, 220 (8.24%) yielded growth. Out of these 220 culture positive urine samples, 68.6% were from female patients while 34.1% from male; male to female ratio of 1:2.2. The mean age of the patients was 40 years, ranging from 2 to 79 years. Gram negative bacteria constituted the major bulk 196 (89%), gram positive bacteria turned out 17 (7.8%) whereas *Candida* spp constituted 07 (3.2%). Frequency of culture positive isolates is shown in Figure 1. The sensitivity pattern of all culture positive urinary isolates is listed in Table 1.

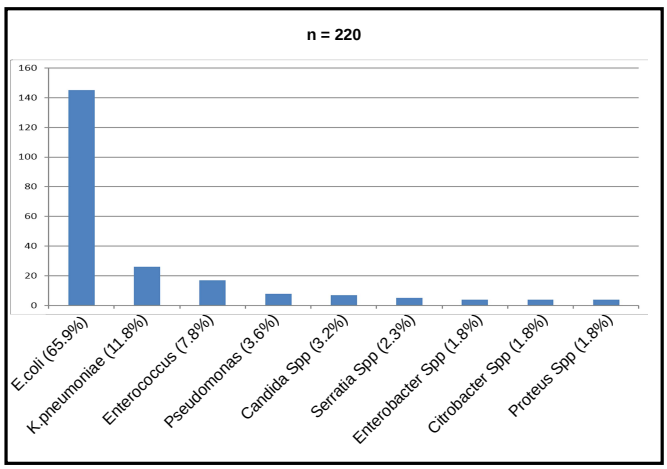

Fig 1. Frequency of culture positive microorganisms isolated from the urine samples

Table 1. Antibiotics showing sensitivity pattern against Gram negative & Gram Positive urinary isolates

Antibiotics	*E.coli* = **145 (%)**	*K.pneumoniae* **= 26 (%)**	*Pseudomonas* **spp = 8 (%)**	*Serratia* **spp = 5 (%)**	*Enterobacter* **spp = 4 (%)**	*Citobacter* **spp = 4 (%)**	*Proteus* **spp =4 (%)**	*Enterococcus* **spp = 17 (%)**
AMP	12(8.3)							6 (35.3)
GEN	85(58.6)	11(42.3)	4(50)	3(60)	1(25)	2(50)	2(50)	
AMI	126 (86.9)	16 (61.5)	6(75)	4(80)	4(100)	3(75)	4(100)	
AUG	31(21.4)	7(26.9)				1(25)		
IMP	134(92.4)	22(84.6)	7(87.5)	5(100)	3(75)	4(100)	4(100)	
TZP	98(67.6)	13(50)	4(50)	4(80)	3(75)	1(25)	3(75)	
SXT	27(18.6)	8(30.8)	1(20)	4(100)	2(50)			
DOX	43(29.6)	11(42.3)			2(50)	3(75)		4(23.5)
CIP	49(33.8)	9(34.6)	5(62.5)	1(20)		2(50)	2(50)	3(17.6)
CRO	44(30.3)	10(38.5)		1(20)		2(50)		
FEP			3(37.5)					
NIT	138(95.2)	11(42.3)			4(100)	3(75)		14(82.3)
FOS	140(96.5)	23(88.5)		4(80)	4(100)	3(75)	4(100)	16(94.1)
TGC	105 (72.4)							
VAN								17(100)
LNZ								16(94.1)

Key: AMP=ampicillin, GEN=gentacin, AMI=amikacin, AUG=amoxicillin/clavulanic acid, IMP=imepenem, TZP=tazobactum/piperacillin, SXT=trimethoprim/ sulfamethoxazole, DOX=doxycyline, CIP=ciprofloxacin, CRO=ceftriaxone, FEP=cefipime, NIT=nitrofurantoin, FOS=fosfomycin, TGC=tigecycline

Discussion

There are an estimated 150 million UTIs per year, worldwide.[17] UTIs result in a significant morbidity and high treatment cost in community. Females seem to be more prone to UTIs most probably because of close proximity of urinary tract with anal canal and short urethra. Effective management of patients suffering from bacterial UTIs is usually dependent on the identification of the bacterial isolate along with selection of an appropriate and effective antibiotic agent used for the treatment of bacterial organisms in question.[18] With the discovery of new era pertaining to antibiotics, newer mutant strains are also developing thus making it more difficult to control the infection especially in developing countries like Pakistan where high-tech diagnostic facilities are sparse.

The present study focused on the local status of antimicrobial susceptibility pattern in uropathogens with a view to monitor the continuous changing pattern of bacterial resistance and to make further improvements to treat UTIs. Female patients constituted the bulk of urinary isolates. *E.coli* turned out to be the most common urinary tract bacteria. Similar studies conducted by Sabir S *et al* at Lahore and by Shahzad KA *et al* at Peshawar, Pakistan also revealed *E.coli* as the most common bacteria accounting for 80% and 77% respectively.[19,20] In our study, *E.coli* showed the highest susceptibility (96.5%) to fosfomycin followed by nitrofurantoin (95.2%), which is consistent with an international study by Fajfr F *et al*.[21] Susceptibility of *E.coli* isolates to imepenem in our study was found to be 92.4%, which is quite comparable with a similar study carried out by Kidwani SS *et al* at Karachi one year ago.[22]

In comparison to a study done by Khan IU *et al* at AFIP three years ago, decreasing trend of susceptibility of trimethoprim/ sulphamethoxazole and ceftriaxone to *E.coli* was observed in our study results, which may be due to injudicious use of these antimicrobials now a days.[23] Regionally in Bangladesh, a recent study by Majumder MI *et al* reported 34.5% *E.coli* isolates susceptible to ciprofloxacin, which is quite comparable to our results, however *E.coli* isolates in our study showed less susceptibility to aminoglycosides and trimethoprim/ sulfamethoxazole.[24] Our study revealed that susceptibility of all uropathogens to ampicillin is the lowest. In our study 34.6% *K.pneumoniae* isolates showed susceptibility to ciprofloxacin which is consistent to an Indian study which showed 34.8% susceptibility.[25] Similarly, our study results showed 87.5% *P.aureginosa* isolates susceptible to imepenem and 75% to amikacin as compared to 88.9% and 77.8% respectively claimed in an Indian study carried out by Kalpana S *et al*, which is also quite comparable.[25] Awareness among the population about UTI, early diagnosis and effective treatment may play a vital role in the management of this disease. The key strategy to control the emergence of multidrug resistance among uropathogens is early and appropriate antimicrobial susceptibility testing and judicious use of antibiotics in appropriate dosage for a specific period of time.

Conclusion

UTI being considered as one of the most prevalent infectious diseases causes morbidity in patients of almost all age groups and gender. Antibiotics like fosfomycin and nitrofurantoin showed excellent susceptibility not only against *Enterobacteriaceae* family but also against gram positive organisms. Vancomycin and linezolid are still good antibiotics reserves to treat multidrug resistant and complicated UTIs caused by *Enterococcus* spp. The ever increasing resistance of uropathogens against oral antibiotics necessitates proper drugs susceptibility testing in order to have base line data to formulate effective antibiotics policy for guidance and accordingly patient treatment.

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