ORIGINAL ARTICLE

Spectrum of Uropathogens and their Antibiotic Susceptibility Pattern – Four Years Data from a Reference Laboratory of Karachi, Sindh.

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Abstract

Background
Urinary tract infections are amongst the most common bacterial infections. It is therefore important to investigate spectrum of uropathogens as well as their antibiotic susceptibility in order to implement appropriate use of antibiotics. The aim of the present study is to investigate common pathogens isolated from urine samples and to reveal antibiotic susceptibility patterns of these pathogens against routinely used antibiotics.

Method
This retrospective study was conducted at the Dow Diagnostic Research and Reference Laboratory during 2009 – 2012. All urine samples collected during the study period were investigated for culture growth and sensitivity patterns. Isolated uropathogens were then tested against antibiotics by Kirbu bur disc diffusion method.

Results
Out of 14,787 urine samples investigated, 4,479 (30%) showed significant bacterial growth. Among those which showed bacterial growth, 2647 (59%) were from females patients while 1832(40.9%) were from male patients. Overall, frequency of gram negative bacteria was higher 3919(87%) compared to gram positive bacteria 560 (12%). Of the gram negative organisms, 2268 (58%) samples were positive for *Escherichia coli* while in the gram positive; *Enterococci species* was most frequent 283 (50.5%). The antibiotic sensitivity profile of the isolated microorganisms showed that Imipenem, Piperacillin/Tazobactam, Amikacin and Fosfomycin were most effective antibiotics for gram negative bacteria and Vancomycin and Chloramphenicol were most effective against gram positive bacteria.

Conclusion
Our data demonstrated that prevalence of urinary tract infection is 30 %. *E.coli* is the most common gram negative bacteria and *Enterocccus* is the most common gram positive bacteria isolated from the investigated urine samples. Antibiotic susceptibility pattern showed that gram negative bacateria are highly sensitive to Imipenem, Piperacillin/ Tazobactam, Amikacin and Fosfomycin and gram positive bacteria are highly susceptible to Vancomycin and Chloramphenical. These antibiotics can be used for empirial treatment of urinary tract infection. It is necessary to have proper and effective use of antibiotics for treatment of UTI to avoid recurrence and emergence of resistant strains.

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Keywords
Urinary tract infection, Gram positive bacteria, Gram negative bacteria, multidrug resistant.

Introduction

Urinary tract infections (UTIs) are considered as one of the frequently reported infections in both outpatients & hospitalized patients.[1] UTIs are identified as persistent presence of actively dividing microorganisms in the urinary tract as well as microbial colonization of the urine.[2,3] Clinical manifestations of UTIs can vary from mild cystitis to pyelonephritis and even septicemia if not appropriately treated.[4] Each year approximately 150 million people suffer from UTIs globally, rendering the disease as one of the major reasons of economic burden, particularly in developing countries.[5] UTIs contribute to major utilization of antimicrobial drugs.[6] Patients suffering from UTIs are usually given antibiotics empirically before the outcome of urine culture and sensitivity report. Therefore there is high chance of development of antibiotic resistance in urinary pathogens due to indiscriminate use of antibiotics.[1] Various research studies have reported about changing pattern of uropathogens and their sensitivity to commonly available antibiotics in the last 20 year due to antibiotic resistance which has now become a major health problem all around the world and varies in different countries.[1] For the effective treatment of the UTIs, it is necessary to have recent data on spectrum of uropathogens causing UTIs and their antibiotic sensitivity pattern in a particular geographical areas.[6] It will help physicians to prescribe most appropriate empirical treatment in critically-ill patients. Taking into account these objectives a retrospective study was conducted to investigate the spectrum of uropathogens in urine samples and their antibiotic susceptibility pattern.

Material & Methods

The study was performed at the Dow Diagnostic Research and

Reference Laboratory (DDRRL), Dow University of Health Sciences (DUHS) Karachi. All urine samples collected during the study period from 2009-2012 were investigated for culture and sensitivity tests followed by investigation of antibiotic resistance pattern. Urine samples were received from both outpatient and inpatient departments. Urine samples were collected in disposable, wide mouth sterile containers. Urine samples were processed on immediate basis after collection in bacteriology section. In case of any delay, the samples were refrigerated at 4-6°C. The samples were examined using direct microscopy for white blood cell count. The samples were inoculated on Cysteinelactose electrolyte deficient media by semi-quantitative plating method using a calibrated loop carrying 0.001ml of urine. The plates were incubated at 37°C for 24 hrs. Cultures without any colony after 24hrs incubation were further incubated for 48hrs before reporting no bacterial growth. Samples with colony count equal or more than 10^5 Cfu/ml were considered positive. Identification and interpretation of cultures of bacterial isolates was done by using standard microbiological method.[1]

Susceptibility testing to antibiotics was performed by disc diffusion method on Mueller Hinton agar (MHA) as recommended by clinical laboratory standard institute (CLSI).[6] Required antibiotics were placed on the inoculated MHA plates according to whether the testing bacteria are gram positive or gram negative. The antibiotic discs used for susceptibility testing included Amoxicillin / Clavulanic acid, Ampicillin, Amikacin, Aztreonam, Imipenem, Piperacillin / Tazobactam, Gentamycin, Ceftriaxone, Cefuroxime, Ofloxacin, Ciprofloxacin, Cotrimaxazole, Cefixime, Ceftazidime, Nitrofurantoin, Pipemidic acid, Fosfomycin, Erythromycin, Tetracycline, Vancomycin, Chloramphenicol, Penicillin, Clindamycin, Fusidic acid & Cloxacillin. These agar plates were then incubated for 24 hrs at 37°C. Inhibition zones were measured in mm. Interpretation of inhibition zone was done according to CLSI guidelines. Statistical analysis of the results was done by SPSS version 16. Descriptive statistic was computed in percentages.

Results

A total 14,787 urine samples were submitted for culture & sensitivity from 2009-2012. Of these, 4,479 urine samples were positive for bacterial growth. Among these, 1832 (4%) samples were of male patients and 2647 (59%) samples were of female patients. The patients were between the age ranges of 1-90 years with mean age 45 years. Frequency of infections was much higher in the patients between 21-30 years of age followed by 51-60 years of age as compared to other age groups (Figure1). The isolated microorganisms from urine samples included both Gram negative as well as Gram positive bacteria with the former being more frequent (87%) (Table1). Of the gram negative bacteria, most frequent isolate was *Escherichia coli (E.coli)* (57.8%) followed by *Klebsiella species (sp.)* (23%), *Enterobacter sp.* (5.6%), *Citrobacter frundii* (4.7%), *Pseudomonas sp*. (3.3%), *Pseudomonas aeruginosa* (3.0%), *Proteus mirabilis* (1.3%), *Proteus vulgaris* (0.4%), *Acinetobacter sp*. (0.4%) and *Citrobacter diversus* (0.2%). In gram positive group most frequent isolate was *Enterococcussp*.(50.5%) followed by

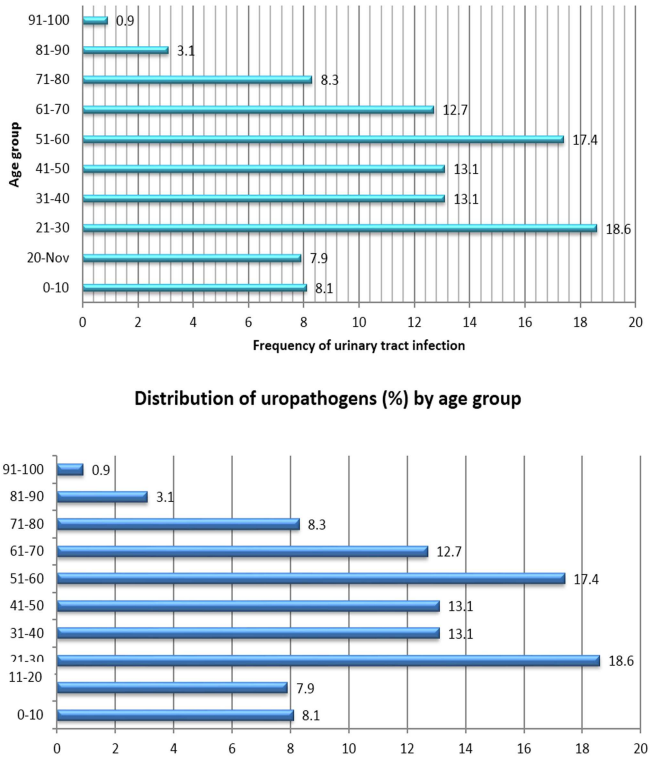

Fig 1. **Distribution of Urinary tract infections (%) by age group in urine samples tested during 2009-2012 at a reference laboratory at Karachi (N=4479)**

Table 1: Bacteria isolated from urine samples during 2009-2012 at a reference laboratory at Karachi (N=4479)

Gram Negative Bacteria(n=3919)		Gram Positive Bacteria(n=560)	
Name of bacteria	**No. of isolate (87%)**	**Name of bacteria**	**No. of isolate (12%)**
Escherichia coli	2268 (57.8%)	*Enterococcus species*	283 (50.5%)
Klebsiella species	890 (22.7%)		
Enterobacter species	223 (5.6%)		
Citrobacter frundii	188 (4.7%)	*Staphylococcus aureus*	177 (31.6%)
Pseudomonas species	132 (3.3%)	*MSSA*	112(63.3%)
Pseudomonas aeruginosa	121 (3.0%)	*MRSA*	65 (36.7%)
Proteus mirabilis	54 (1.3%)	*Streptococcus species*	100 (17.8%)
Proteus vulgaris	17 (0.4%)		
Acinetobacter species	16 (0.4%)		
Citrobacter diversus	10 (0.2%)		

Staphylococcus aureus (32%) & *Streptococcus sp.* (18%). Imipenem and Tazobactam were the most susceptible antibiotics for *Enterobacteriaceae* (Table 2). Imipenem and Amikacin were found to be most sensitive antibiotics for non-fermenters (Table 3). Antimicrobial susceptibility pattern of gram positive bacteria, revealed Vancomycin and Chloramphenicol displaying maximum susceptibility (Table 4).

Discussion

Antimicrobial drug resistance is a major health problem faced by the patients due to irrational use of antimicrobial drugs. The epidemiology and susceptibility profile of uropathogens varies in different regions as well as within the same region over a period of time. Regular surveillance of antimicrobial resistance pattern is necessary both globally and at local level.[7] In our study frequency of urinary tract infections (UTIs) was found to be 30.2% which is in accordance with the results of study done by Chongtham U. *et al*[8] (30%) but in contrast to Kulsoom B. *et al*[2] (74.10%) & Prakash D. *et al* (53.8%)[9]. There could be several reasons of disparity found in the frequency of uropathogens isolated in different studies such as duration of study, urine culture methodology, varying population size, diverse population, variable level of personal hygiene and environmental factors.[10, 11] Our data demonstrated that UTIs were more common in women compared to men. These results are in line with the previous studies conducted by Shilpi T. *et*

Table 3: Antimicrobial susceptibility pattern of non-fermenters isolated from urine samples during 2009-2012 at a reference laboratory in Karachi(N=4479)

Antibiotics	Acinetobacter species (n=16)	Pseudomonas species (n=132)	Pseudomonas aeruginosae (n=121)
Amoxicillin	**64.5***	-	-
Ampicillin	9.5	-	-
Amikacin	**83.0***	**71.2***	**74.4***
Aztreonam	53.5	36.4	49.6
Imipenem	**92.5***	**78.8***	**87.6***
Pipericillin/ Tazobactem	21.5	**76.5***	**81.0***
Gentamycin	21.5	49.2	**62.0***
Ofloxacin	9.0	53.0	53.7
Ciprofloxacin	7.0	-	-
Cotrimaxazole	18.0	-	-
Cefexime	16.0	-	-
Ceftazidime	46.0	52.3	51.2
Nitrofurantoin	22.5	-	-
Pipemidic acid	27.5	-	-
Ceftriaxone	11.0	-	-
Cefuroxime	14.0	-	-
Fosfomycin	28.0	-	-

*Microorganisms showing significant sensitivity to antibiotics.

Table 2: Antimicrobial susceptibility pattern of *Enterobacteriaceae* isolated from urine samples during 2009-2012 at a reference laboratory in Karachi (N=4479)

Antibiotics	Escherichia coli (n=2268)	Klebsiella species (n=890)	Enterobacter species (n=223)	Proteus Mirabilis (n=54)	Proteus vulgaris (n=17)	Citrobacter diversus (n=10)	Citrobacter frundi (n=188)
Amoxicillin	43.6	38.4	45.7	53.7	11.8	40.0	29.3
Ampicillin	11.9	3.3	23.8	18.5	11.8	20.0	9.6
Amikacin	**84.0***	**74.6***	**77.1***	**68.5***	53.0	**100***	**72.3***
Aztreonam	42.6	39.1	37.7	50.0	53.0	40.0	40.4
Imipenem	**98.3***	**94.7***	**93.3***	**94.4***	**70.6***	**100***	**88.3***
Pipericillin/ Tazobactem	**79.0***	**72.5***	**80.7***	**96.3***	**70.6***	**90.0***	**72.3***
Gentamycin	54.7	55.7	**59.9***	51.9	35.3	**70.0***	53.2
Ofloxacin	32.9	54.4	48.9	44.4	47.1	50.0	44.7
Ciprofloxacin	32.8	52.4	30.9	27.8	41.2	50.0	37.2
Cotrimaxazole	24.6	35.4	33.6	16.7	11.8	30.0	34.0
Cefexime	32.9	34.0	33.6	31.5	17.6	20.0	23.4
Ceftazidime	39.5	45.4	53.8	50.0	29.4	**60.0***	53.2
Nitrofurantoin	**81.4***	37.4	55.2	16.7	11.8	**80.0***	34.0
Pipemidic acid	19.6	37.8	18.8	24.1	17.6	30.0	23.4
Ceftriaxone	38.3	40.9	50.7	50.0	41.2	**60.0***	50.0
Cefuroxime	33.6	36.6	39.9	42.6	11.8	40.0	34.6
Fosfomycin	**63.5***	**64.6***	**67.3***	48.1	41.2	**70.0***	**59.0***

*Microorganisms showing significant sensitivity to antibiotics.

Table 4: Antibiotic sensitivity pattern of gram positive bacteria isolated from urine samples during 2009-2012 at a reference laboratory in Karachi

	Gram Positive Bacteria		
Antibiotics (N=4479)	**Enterococci species (n=283)**	**Stapylococci aureus (n=177)**	**Streptococci species (n=100)**
Erythromycin	23.3	35.6	44.0
Amoxicillin	**78.8***	-	-
Ampicillin	**71.7***	-	-
Amikacin	-	**84.7***	-
Clindamycin	-	**61.0***	37.0
Fusidic acid	-	57.6	-
Cloxacillin	-	36.7	-
Gentamycin	37.5	**62.7***	-
Ofloxacin	41.7	44.6	-
Ciprofloxacin	43.8	-	-
Cotrimaxazole	9.9	45.2	-
Tetracyclin	19.8	42.9	-
Vancomycin	**84.8***	**88.1***	**87.0***
Chloramph-enicol	**70.7***	**66.1***	**74.0***
Penicillin	58.0	23.7	**66.0***
Nitrofurantoin	**69.6***	-	-
Pipemidic acid	5.7	-	-
Fosfomycin	**73.9***	-	-
Ceftriaxone	-	-	**62.0***

*Microorganisms showing significant sensitivity to antibiotics.

al[12] & Somashekara SC *et al.*[13] The reason behind high prevalence of UTI in females is due to shorter urethra, sexual activity, urinary incontinence & close proximity of the urethral meatus to anus.9,14 In this study, gram negative bacilli constituted 87% of the total isolates while gram positive constituted 12%. *E.coli* (57.8%) was found to be most predominant isolated microorganism among the gram negative bacilli followed by *Klebsiella sp.* (22.7%) which has also been reported in the studies conducted by Paryani J. P. *et al*[15], & Chongtham U. *et al.*[8] & Lakshminarayana SA *et al.*[16] *Enterococcus sp.*(50.5%) was found to be the most common etiological agent amongst the gram positive bacteria. This is in contrast to study done by Manikandan S. *et al*[17] according to which *Staphylococcus aureus* was the most common isolated gram positive bacteria. In the current study UTIs were significantly higher in patients between 21-30 years of age which is in accordance with the study conducted by Kulsoom B. *et al*[2] and Bitew A. *et al.*[11] However the second commonly affected age group was found to be patients having age between 51- 60 years which has also been reported in a study by Nwadioha S. I. *et al.*[18] Literature search showed that elderly males have a higher incidence of UTIs compared to elderly females.9 According to the results of antibiotic susceptibility, the most sensitive antibiotic against *Enterobacteriacea* was Imipenem and Tazobactam followed by Amikacin and Fosfomycin. It is consistent with the results of the study by Yadav M. *et al*[19] and Niranjan V*et al.*20 Among the non-fermenters, Imipenem and Amikacin were found to be most sensitive antibiotics. These findings were also reported by Yadav M. *et al,*[19] Shilpi T. *et a,*[12] Prakash D. *et al*[9] and Paryani J.P. *et al.*[15] Among the gram positive bacteria, Vancomycin and Chloramphenicol were the most sensitive antibiotics. Same results are reported by Yadav M. *et al*[19] and Bitew A. *et al*[11] and Kashef N. *et al.*[12]

Low level of resistance was noticed against Aminoglycoside drugs in both gram negative and gram positive organisms and hence may be useful in empirical treatment of UTI. Patients have limited access to Aminoglycosides as it is available in injectable form and therefore displayed low resistance rates.[19] However high resistance was seen against third generation Cephalosporin, Ampicillin, Amoxicillin, Ciprofloxacin and Cotrimoxazole. This high proportion of resistance is due to easy availability, indiscriminate use of antibiotics in both community and hospital sectors. Moreover non-evidence based unchecked doctor's prescription practices and non-compliance of patients are also prime reasons of emergence of antibiotic resistance.[22] Limitations of study are that patient data (symptomatic vs asymptomatic or critically ill vs stable patients) is not available as the study is retrospective. Source of the patients is not known whether hospitalized or community based. Moreover source of the urine sample is also not present in the data if it is catherized or midstream urine. Further how many samples are from the same patients is not noted for the indication of colonization .

Conclusion

The current study displayed that Imipenem, Piperacillin/Tazobactam and Amikacin were the most effective drugs against Gram-negative bacteria while Chloramphenicol and Vancomycin were the most sensitive drugs against Gram-positive bacteria.

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