Original Research Article

Spectrum and antimicrobial susceptibility pattern of Uropathogens: Indoor versus outdoor isolates.

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ABSTRACT

Background & objectives: The resistance of uropathogens to commonly prescribed antimicrobials is increasing globally. As the susceptibility of uropathogens varies according to place and time, the present study was undertaken to know the local epidemiology and antimicrobial susceptibility patterns (AMSP) of common bacterial uropathogens.This helps in formulating effective empirical treatment.

Method: A total of 3352 consecutive urine specimens over a period of one year in a tertiary care hospital in Western India were cultured by semiquantitative method.The pathogens isolated were identified by standard methods and their antimicrobial susceptibility was done by Kirby Bauer disk diffusion method as per CLSI guidelines.The data was analysed by using WHONET 5.6 software.

Results : Out of 3353 urine samples, 63% were sterile, 24% showed significant growth, 5.27 % showed insignificant growth and 7.45 % were collection contaminants.A total of 988 bacterial isolates comprising of 814 (82 %) Gram negative bacilli (GNB) and 174 (18 %) Gram positive cocci (GPC) were isolated.Amongst GNB low sensitivity was observed to Ampicillin (OPD-7%,IPD -6%),Cotrimoxazole (OPD- 30%,IPD-29%),fluroquinolones like norfloxacin (OPD-44%,IPD-31%) and cephalosporins like cefotaxime (OPD-22%,IPD-14%) and cefepime (OPD-39%, IPD-33%). Comparatively higher sensitivity was observed to nitrafurantoin (OPD-87%,IPD-65%),aminoglycosides like amikacin (OPD -72% ,IPD-58%) and gentamicin (OPD-61%,IPD-53%),followed by Piperacillin tazobactum (OPD-72%,IPD-59%) and Meropenem (OPD-70%), IPD – 52%).ESBL Production was observed amongst 40% of *Escherichia coli* and 60% of *Klebiella pneumoniae*. Amongst GPC,36% MRSA and 2% VRE were observed in only indoor patients.Gram positive isolates showed low sensitivity to fluroquinolones like Norofloxacin (OPD- 30%,IPD- 26%), ciprofloxacin (OPD-30%, IPD-21%) and tetracyline (OPD- 52%,IPD- 62%). Higher sensitivity was observed to vancomycin (OPD-100%,IPD-98%),teicoplanin(OPD-100%,

IPD-98%) and linezolid (OPD and IPD -100%).

Conclusion- Local epidemiology and susceptibility pattern of uropathogens should be studied to formulate effective empirical treatment regimen.

Key words*: Uropathogens*, *AMSP, MDR*

1. INTRODUCTION

Urinary tract infections (UTI) is one of the most common infections observed in clinical practice among community and hospitalized patients. UTI forms 40 - 50% of the total nosocomial infections.Urinary tract infection often results in serious complications like secondary bacteremia and sepsis leading to a rise in mortality [1].

The favorable chemical composition of human urine can support the growth of several different strains of bacteria. *E. coli* is the cause of 80–85% of urinary tract infections, with Enterococcus species being the other main cause. Other bacterial species that causes the UTI include *Klebsiella* , *Proteus*, *Pseudomonas*, and *Enterobacter*. UTI may also be due to fungal or viral infections, although these are uncommon and typically related to abnormalities of the urinary system or urinary catheterization. Urinary tract infections due to *Staphylococcus aureus* typically occurs secondary to blood borne infections [2,3].

So this study was carried out to determine the prevalent uropathogens in our hospital and their antimicrobial susceptibility pattern to commonly used antimicrobials in order to formulate an antibiotic policy for empirical treatment. We also compared the antibiotic sensitivity pattern of the bacterial isolates between outpatients and inpatients. Formulation of effective empirical treatment gives appropriate treatment and inaddition helps preventing drug resistance by avoiding inappropriate and indiscriminate antibiotics usage.

2. MATERIAL AND METHODS:

A prospective observational study was carried out in the Bacteriology laboratory of the department of Microbiology from Jan 2016 to December 2016. Urine samples were received from various outpatient Departments (OPDs) and Inpatient Departments (IPDs) of a tertiary care hospital. Clean catch, midstream urine samples were collected in sterile universal containers and immediately transported to laboratory and processed..The samples were plated on CLED agar by the semiquantitative plating method using the calibrated loop technique (0.001 mL). Plates were incubated aerobically overnight at 37°C.Pure growth of an isolate in a count of $\geq 10^5$colony forming units (CFU) per milliliter of midstream voided urine was considered as significant bacteriuria. Counts of 10^4 and below were considered as Insignificnt growth.Growth of ≥3 isolates in a sample was considered as collection contamination[CDC reference] .Conventional methods of identification were used for identification of the bacterial isolates [4]. Antimicrobial susceptibility test (AST) was done on Mueller Hinton agar (Himedia Labs Ltd) by the KirbyBauer technique according to the CLSI guidelines 2016 [5]. Data was entered in WHONET 5.6 and was analysed to know significant difference between sensitivity of OPD and IPD isolates Chi square test and fisher's exact test were used. Yate's correction was applied wherever necessary,P value <0.05 was considered significant.

RESULTS

A total of 3353 consecutive urine samples were included in the study. Of these, 2114 (63%) were sterile, 812 (24%) showed significant growth, 177 (5.27 %) showed insignificant growth and 250 (7.45%) were collection contaminants and were repeated after proper collection.
The 812 samples with significant growth yielded 988 bacterial isolates with 814(82%) Gram negative bacilli (GNB) and 174 (18%) Gram positive cocci (GPC).
The distribution of Gram positive isolates (table 1) along with their antibiotic sensitivity pattern (table 2) in both OPD and IPD setup revealed that majority of UTI infections caused by Gram positive cocci were due to *Enterococcus spp*, followed by *Staphylococcus aureus* in both OPD and IPD patients. CONS were obtained from only indoor patients.

Table no.1: Distribution of Gram positive isolates in UTI (n=174)

Sr. No	Isolate	OPD (n=23) No %	IPD (n=151) No. %	Total (n= 174)
1	*Enterococcus spp*	18 (78.26%)	136 (90%)	154
2	*Staphylococcus aureus*	5 (21.74%)	11 (7%)	16
3	*Coagulase negative staphylococci*	-	4 (3%)	4

Methicillin resistance was found in 36% of the *Staphylococcus aureus* isolates from IPD (4 out of 11 isolates) however no MRSA was isolated from OPD patients. Percentage of high level aminoglycoside resistance and Vancomycin resistance in Enterococcus isolates from IPD was 58% (78 of 136 isolates) and 2% (3 of 136 isolates) respectively. High level aminoglycoside resistance in OPD patients was 50% (9 of 18 isolates) and no VRE was isolated from OPD Patients.

Table no 2: Antimicrobial sensitivity of Gram positive isolates (n=174)

Sr. No	Antibiotics	OPD (% of sensitivity) (n=23)	IPD (% of sensitivity) (n=151)	P value
	1st Line drugs			
1	Penicillig G	05 (22%)	23(15%)	P value-0.42 (NS)
2	Norfloxacin	07(30%)	39(26 %)	P value-0. 64 (NS)
3	Nitrafurantoin	20 (87%)	125(83%)	P value-0.88 (NS)
4	Ciprofloxacin	07 (30 %)	32 (21 %)	P value-0.32 (NS)
5	Tetracycline	12 (52%)	94 (62 %)	P value-0.35 (NS)
	2nd Line drugs			
6	Vancomycin	23(100%)	148 (98%)	P value-0.5(NS)
7	Teicoplanin	23(100%)	148 (98%)	P value-0.5(NS)
8	Linezolid	23 (100%)	151 (100%)	P value-1.00(NS)

Antimicrobial sensitivity of Gram positive isolates for all antibiotics among OPD and IPD showed similiar pattern and the difference was not stastically significant.
Amongst gram positive isolates, there was lower sensitivity to fluroquinolones like Norofloxacin (30% in OPD isolates and 26% in IPD), ciprofloxacin (30% in OPD and 21% in IPD isolates) and tetracyline (52% in OPD and 62% in IPD isolates). Gram positive isolates showed higher sensitivity to teicoplanin (100%OPD and 98% IPD) and Linezolid (100% OPD and IPD). There was higher sensitivity to orally administered antimicrobials like nitrofurantoin in both OPD (87%) and IPD (83%) isolates. Antimicrobial sensitivity of Staphylococcus isolates to cotrimoxazole was 40%(2/5 isolates) in OPD and 87%(13 of 15 isolates) in IPD isolates and to gentamicin was 0% (0/5 isolates) in OPD and 87% (13 /15 isolates) in IPD isolates.

Table no. 3: Distribution of Gram negative isolates in UTI (n=814)

Sr. No	Name of isolate	OPD (n=121) No. %	IPD (n= 693) No. %	Total
1	*Escherichia coli*	60 (50%)	347(50%)	407
2	*Klebsiella pneumoniae*	20 (17%)	90(13%)	110
3	*Enterobacter spp*	20 (17%)	60 (9%)	80
4	*Citrobacter spp*	3 (2%)	30 (4%)	32
5	*Pseudomonas aeruginosa*	8 (7%)	89 (12%)	97
6	*Acinetobacter spp*	7 (6%)	40 (8%)	47
7	*Other Non fermenterGNB*	3 (2%)	20 (3%)	23
8	*Proteus spp*	-	17 (2%)	17
9	Total	121	693	814

The distribution of gram negative isolates (table 4) revealed that *E.coli* (50%) was predominant isolate in OPD set-up followed by *Klebsiella pneumoniae* (17%), and *Enterobacter spp* (17%). In IPD patients *E.coli* was the predominant isolate (50%) followed by *Klebsiella pneumoniae* (12%) and *Pseudomonas aeruginosa* (12%).Also there is higher percentage of nonfermenters in IPD patients.

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Sr. no.	Antibiotic	OPD (% of sensitivity) (n=121)	IPD (% of sensitivity) (n=693)	P value P<0.001 – Stastically significant
	1st Line drugs			
1	Amikacin	87 (72%)	401 (58%)	P value =0.003 (HS)
2	Ampicillin	07 (7 %)	42 (6%)	P value =0.81 (NS)
	Nitrofurantoin	105 (87%)	453 (65%)	P value =0.0001 (VHS)
3	Tetracycline	63 (52%)	311 (45%)	P value =0.143 (NS)
4	Gentamicin	74 (61%)	367 (53%)	P value =0.09 (NS)
5	Norfloxacin	53 (44%)	214 (31%)	P value =0.005(S)
6	Cefotaxime	27(22%)	97 (14%)	P value =0.01 (NS)
7	Cotrimoxazole	36 (30%)	200 (29%)	P value =0.84 (NS)
	2nd Line drugs			
8	Meropenem	85(70 %)	360 (52%)	P value =0.0001 (HS)
9	Cefoperazone sulbactum	83 (69%)	311 (45%)	P value =0.0001 (HS)
10	Piperacillin tazobactum	87 (72%)	408 (59%)	P value =0.006 (S)
11	Cefepime	47 (39%)	228 (33%)	P value =0.20(NS)
12	Aztreonam	47 (39%)	152 (22%)	P value=0.0006(HS)

On comparing antimicrobial sensitivity of Gram negative isolates from OPD and IPD setup, OPD isolates were more senitive and the difference is stastically significant for antimicrobials like amikacin, nitrofurantoin,norfloxacin, meropenem,cefaprazone sulbactum, piperacillin tazobactum and aztreonam.

In our study, there was low sensitivity of gram negative isolates to ampicillin (7% in OPD and 6% in IPD patients), cotrimoxazole (30% in OPD and 29% in IPD patients), norfloxacin (44% in OPD and 31% in IPD Patients) and cephalosporins like cefotaxime (22% in OPD and 14% in IPD) and cefepime (39%in OPD and 33% in IPD).Comparatively higher sensitivity observed to Nitrofurantoin (87%in OPD and 65% in IPD isolates),aminoglycosides like amikacin (OPD -72% , IPD – 58%) and gentamicin (OPD -61% , IPD – 53%),followed by Piperacillin tazobactum (OPD -72% , IPD – 59%) and Meropenem (OPD -70% , IPD – 52%).

DISCUSSION

This study provides valuable data to compare and monitor the status of antimicrobial resistance among uropathogens to improve efficient empirical treatment. Increasing antimicrobial resistance among uropathogens has been documented globally. In our study, 24% of isolates showed significant bacteriuria, which is comparable to other Indian studies like Mandal etal [8] and Lakshmi etal [1] showing significant bacteruria as 26.01% and 23.85% respectively.

In our study amongst the gram negative bacteria, *Escherichia coli* is the predominant pathogen followed by *Klebsiella pneumoniae* and other enterobacteriaciae. This is in consistence with findings of other studies in which *E.coli* and other enterobacteriaciae were the most frequently reported uropathogens [1],[2],[3],[6],[7]. Enterobacteriaceae have several factors responsible for their attachment to the uroepithelium. These gram negative aerobic bacteria colonize the urogenital mucosa with adhesion, pili, fimbriae, and P1blood group phenotype receptor [6]. In our study, Enterobacteriaceae bacteria accounted for 65.38% of all the isolates (646/988 isolates).
Our study reveals 40 % of the *E. coli* isolates and 60% of *Klebsiella spp* were ESBL producers. Aggarwal et al. reported 40% of E. coli and 54.54% of Klebsiella species from uropathogens to be ESBL producers from Rohtak,Haryana[7]. In another study from Rajasthan, Dalela etal reported 73% of *Ecoli* and 59% of *Klebsiella species* from uropathogens to be ESBL Producers [3]. This geographical difference may be due to different patterns of antibiotic usage. Our study confirms the global trend towards increased resistance to β lactam antibiotics. ESBL producing bacteria may not be detectable by routine disk diffusion susceptibility test, leading to inappropriate use of antibiotics and treatment failure. It is emphasized that institutions should employ appropriate tests for their detection and avoid indiscriminate use of third generation cephalosporins.

Methicillin resistance was found in 36% of the *Staphylococcus aureus* isolates from IPD. Dalela etal reported overall prevalence of MRSA in uropathogens as 42.4% [3]. Aggarwal et al also reported prevalance of MRSA in uropathogens as 36.84% [9]. Emergence of 2% VRE in IPD set-up is alarming and emphasizes importance of infection control measures to control its spread and transfer of vancomycin resistance to staphylococci.Mandall et al has reported 3.2% VRE in uropathogens [8].

In our study there is low sensitivity of gram negative isolates to oral antimicrobials like Ampicillin (7% in OPD and 6% in IPD patients) and Cotrimoxazole (30% in OPD and 29% in IPD patients). Similarily gram positive isolates from OPD setup show only 40% sensitivity to cotrimoxazole.These findings are in consistence with the recent data reported from other developing countries. [1,3,8,10]. The high antibiotic resistance against ampicillin and

cotrimoxazole could be attributed to their wide usage for a variety of other indications and is a matter of concern and their use as empirical treatment should be stopped.

Fluoroquinolones have a wide variety of indications, they permeate most body compartments, and are ubiquitously prescribed, accounting for the emergence of their resistance. In our study amongst gram negative bacteria only 44% OPD isolates and 31% IPD isolates were sensitive to Norfloxacin.Similarily amongst gram positive cocci, only 29 % OPD isolates and 26% IPD isolates were sensitive to Norfloxacin.Also ciprofloxacin resistance in Gram positive cocci is 27% in OPD and 21% in IPD patients. This increasing resistance to fluroquinolones is also documented in other studies [1,8,10]. Our findings indicate that urgent strategies to counteract increased resistance to these drugs must be developed or their use in uncomplicated infections should be strictly curtailed.In the present study a good sensitivity to Nitrofurantoin amongst Gram positive isolates (OPD – 86% and IPD 83%) and Gram negative isolates (87% in OPD and 65% in IPD patients) was observed. Our findings are similar to other Indian studies which have also demonstrated nitrofurantoin as an appropriate agent for firstline treatment of community acquired UTIs [1,8,10]. Given the fact that Nitrofurantoin has no role in the treatment of other infections, it can be administered orally and is highly concentrated in urine; it may therefore be the most appropriate agent for empirical use in uncomplicated UTI.

Aminoglycosides being injectables are used restrictively in the community care setting and hence have shown better sensitivity rates. Amongst gram negative isolates sensitivity of 72% and 61% to amikacin and gentamicin respectively in OPD patients.Also Staphylococcus isolates from OPD setup showed 100% sensitivity to gentamicin. Sensitivity to cefoperazone/sulbactam and piperacillin/tazobactam was high in OPD isolates 69% and 72% respectively, probably due to their lower usage for treatment of community acquired infections,however sensitivity to cefoperazone/sulbactam and piperacillin/tazobactam amongst IPD isolates was low 45% and 59% .

So recommendations based on findings of our study in our set up are for uncomplicated non-hospitalised patients nitrofurantoin is the best antimicrobial. For complicated Urinary tract infections or serious hospitalized patients aminoglycosides,or BL-BLI agents like piperacillin/tazobactum and cefaperazone sulbactum can be effective.Carbapenems should be reserved for very serious hospital acquired infections.

CONCLUSION

Among the oral drugs norfloxacin,tetracyline and co-trimoxazole should no longer be considered as the first line drugs for the empirical treatment of UTI. Nitrofurantoin can be safely used for un-complicated UTI. Parentral drugs such as aminoglycosides, and Beta lactum and beta lactum inhibitor combination agents like piperacillin/tazobactum, cefaperazone-sulbactum can be the alternative choice for complicated UTI.Carbapenems should be reserved for very serious life threatening infections.Escalation or descaltation of antibiotics should be done as per sensitivity pattern. Also, control measures which include the judicious use of antibiotics, antibiotic cycling, the implementation of appropriate infection control measures and the formulation of an antibiotic policy must be done, to prevent the spread of these strains.It is essential to test and report ESBLs, Vancomycin resistance in entrococcus and MRSA production along with the routine susceptibility testing, which will help the clinicians in prescribing proper antibiotics.

COMPETING INTERESTS – NIL

REFERENCES:

1) Antibiotic Susceptibility Pattern of Uropathogens Isolated in a Rural Teaching Hospital in South India.Lakshmi PV, Leela KS. *Int.J.Curr.Microbiol.App.Sci* (2015) 4(6): 160-167.
2) Antibiotic Susceptibility Patterns of Bacteria among Urinary Tract Infection Patients in Chittagong, Bangladesh.Chowdhury S, Parial R.SMU Medical Journal. (2015) 2(1): 114-126.
3) Antibiotic Resistance Pattern in Uropathogens at a Tertiary Care Hospital at Jhalawar with Special Reference to Esbl, AmpC b-Lactamase and MRSA Production. Dalela G, Gupta S, Jain DK, Mehta P. Journal of Clinical and Diagnostic Research. 2012 May (Suppl-2), Vol-6(4): 645-651.
4) Guidelines for the Collection,Transport,Processing, Analysis and reporting of cultures from specific specimen sources . In: Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Color atlas and textbook of diagnostic microbiology. 6 th ed. Philadelphia: Lippincott; 2006. p. 82-86.
5) CLSI Guidelines 2017. M100 S-27th edition. Clinical Laboratory Standards Institute Performance standards for Antimicrobial Suseptibility testing:1-148.
6) Antibiotic resistance pattern of community acquired uropathogens at a tertiary care hospital in Jaipur, Rajasthan. Sood S, Gupta R.Indian journal of Community medicine. 2012 .37 (1): 39-44
7) Detection of Extended spectrum b lactamase production among uropathogens.Aggarwal R, Chaudhary U, Sikka R.J Lab Physicians 2009 Jan-jun; 1(1): 7-10.
8) Antibiotic resistance pattern among common bacterial uropathogens with a special reference to ciprofloxacinresistant *Escherichia coli.* Mandal J, Acharya NS, Buddhapriya D & Parija SC.Indian J Med Res 136, November 2012 :842-849
9) Prevalance of MRSA as uropathogen in a teaching Tertiary care hospital of North India.Aggarwal R, Goel U,Chaudhary U etal. Int. J.Pharm. Med. & Bio.Sc.(2013) 2(2):18-22.
10) Changing trends in the spectrum of antimicrobial drug resistance pattern of uropathogens isolated from hospitals and community patients with urinary tract infections in Tumkur and Bangalore.Manjunath GN, Prakash R, Vamseedhar Annam , Kiran Shetty Int J Biol Med Res. 2011; 2(2): 504 – 507

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