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Spectrum and Antimicrobial Susceptibility Pattern of Uropathogens: Indoor Versus Outdoor Isolates

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Authors' contributions

This work was carried out in collaboration between all authors. Author MP designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors SM and VD managed the analyses of the study. Author RB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background and 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.

Methods: This is a prospective observational study, where a total of 3353 consecutive urine specimens over a period of one year in a tertiary care hospital 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 Clinical Laboratory Standards Institute (CLSI) guidelines. The data was analyzed by using WHONET 5.6 software.

Results: Of the total 3353 samples, 63% were sterile, 24% showed significant growth, 5.27% showed insignificant growth and 7.45% were collection contaminants. The 812 samples with significant growth yielded 988 bacterial isolates with 814 (82%) gram negative bacilli (GNB) and 174 (18%) gram positive cocci (GPC). Gram negative uropathogens had low susceptibility to

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ampicillin, cotrimoxazole, norfloxacin and cephalosporins. They had good susceptibility to nitrofurantoin and aminoglycosides like amikacin, gentamicin followed by piperacillin-tazobactam and meropenem. ESBL production was observed amongst 40% of *Escherichia coli* and 60% of *Klebiella pneumoniae* isolates. Similarly gram positive uropathogens had low susceptibility to fluoroquinolones like norfloxacin, ciprofloxacin and tetracycline. They had good susceptibility to vancomycin, teicoplanin, linezolid and nitrofurantoin. Amongst the isolates from In Patient department (IPD) 36% of *Staphylococcus aureus* were MRSA and 2% of *Enterococcus* were Vancomycin Resistant *Entercoccus* (VRE).

Conclusion: Local epidemiology and susceptibility pattern of uropathogens should be studied to formulate effective empirical treatment regimen. Our study recommends use of Nitrofurantoin as best antimicrobial for UTI in uncomplicated, non-hospitalised patients. And use of aminoglycosides, or β lactam - βlactamase inhibitor combination agents like piperacillin/tazobactum and cefaperazone-sulbactam in complicated and serious hospitalized patients.

Keywords: Uropathogens; AMSP; MDR.

1. INTRODUCTION

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

UTIs are the fourth most common type of Healthcare-Associated Infections (HAUTIs) [2]. HAUTIs are some of the most-frequently occurring Healthcare Associated Infections (HAIs). In a recent U.S. wide multistate point prevalence survey, 12.9% of all HAI were due to HAUTI [3,4]. In a European point prevalence survey conducted by the European Center for Disease Prevention and Control (ECDC), HAUTI accounted for 19.0% of all HAI [3,5]. Virtually all healthcare-associated UTIs are caused by instrumentation of the urinary tract.

The community acquired Urinary tract infections are mainly uncomplicated, and are mainly caused by *Escherichia coli* as they are normal flora of human intestine and therefore easily colonize the urinary tract. Uncomplicated UTIs in healthy women have an incidence of 50/1000/year [6]. An estimated 50% of women experience at least one episode of UTI at some point in their lifetime and between 20% and 40% of women have recurrent episodes [7,8]. Approximately 20% of UTIs occur in men [9].

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 [10,11].

The introduction of antimicrobial therapy has contributed significantly to the management of UTIs, however the main problem with current antibiotic therapies is the rapid emergence of antimicrobial resistance in hospitals and the community due to rampart and indiscriminate use of antibiotics. This study was carried out to determine the prevalent uropathogens with their antimicrobial susceptibility pattern to commonly used antimicrobials to formulate an effective antibiotic policy for empirical treatment in our community and hospital setup. We also compared the antibiotic sensitivity pattern of the bacterial isolates between outpatients and inpatients. Formulation of effective empirical treatment gives appropriate treatment and in addition helps preventing drug resistance by avoiding inappropriate and indiscriminate antibiotics usage.

2. MATERIALS 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 and urine from catheterized patients were collected in sterile universal containers and

immediately transported to laboratory and processed. The samples were plated on Cystine lactose electrolyte deficient (CLED) agar by the semi quantitative plating method using the calibrated loop technique (0.001 mL). Plates were incubated aerobically overnight at 37°C. In voided midstream urine sample depending upon the number of the colonies grown on the CLED medium, the urine cultures were interpreted as "insignificant" (<10 colonies corresponding to 10^3 colony count), and "significant" (≥100 colonies corresponding to 10^5 colony count). Urine cultures with doubtful significance (>10 - <100 colonies corresponding to 10^4-10^5 colony count) were repeated and interpreted with clinical correlation [12,13]. However, in catheterized patients, colony count of >10^3CFU/ml was considered as significant bacteriuria. Mixture of more than 2 organism types with no predominating organism was reported as grossly contaminated [2]. Conventional methods were used to identify the bacterial isolates [14]. Antimicrobial susceptibility test (AST) was done on Mueller Hinton agar (Himedia Labs Ltd), by the Kirby Bauer disc diffusion technique, according to the Clinical Laboratory Standards Institute (CLSI) guidelines 2016 [15].

Data was entered and analyzed in WHONET 5.6 software. Chi square test and fisher's exact test were used to analyze statistical significant difference between sensitivity of OPD and IPD isolates. Yate's correction was also applied wherever necessary and a P value of <0.05 was considered significant.

3. RESULTS

A total of 3353 consecutive urine samples were included in the study. Out of which, 63% (2114) were sterile, 24% (812) showed significant growth, 5.27% (177) showed insignificant growth and 7.45% (250) were collection contaminants. The 812 samples with significant growth yielded 988 bacterial isolates with 82% (814) gram negative bacilli (GNB) and 18% (174) gram positive cocci (GPC).

Majority of UTI infections caused by gram positive cocci were due to *Enterococcus*, followed by *Staphylococcus aureus*. Antimicrobial sensitivity of gram positive isolates for all antibiotics among OPD and IPD showed similar pattern and the difference was not statistically significant. In our study gram positive uropathogens had low susceptibility to fluoroquinolones like norfloxacin, ciprofloxacin and tetracycline. They had good susceptibility to vancomycin, teicoplanin, linezolid and nitrofurantoin.

Enterococcus species has intrinsic resistance to cotrimoxazole and low level aminoglycoside resistance, hence these agents are neither tested nor reported for *Enterococcus* isolates [15]. Antimicrobial sensitivity of *Staphylococcus* isolates to cotrimoxazole was 40% (2/5 isolates) in OPD and 87% (13/15 isolates) in IPD isolates and to gentamicin was 0% (0/5 isolates) in OPD and 87% (13 /15 isolates) in IPD isolates.

Table 1. Distribution of gram positive isolates in UTI (n=174)

No.	Isolate	OPD (n=23)	IPD (n=151)	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

Table 2. Antimicrobial sensitivity of gram positive isolates (n=174)

No.	Antibiotics	OPD (n=23) (% of sensitivity)	IPD (n=151) (% of sensitivity)	P value
		1st Line drugs		
1	Penicillin G	05 (22%)	23(15%)	P value-0.42 (NS)
2	Norfloxacin	07(30%)	39(26 %)	P value-0. 64 (NS)
3	Nitrofurantoin	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)

Also, urinary tract infections by multi drug resistant gram-positive cocci is more in IPD patients as compared to OPD patients with infection by MRSA contributing to 36%, High Level Aminoglycoside Resistance (HLAR) and Vancomycin Resistance amongst Enterococcus (VRE) as 58% and 2% respectively. Emergence of Vancomycin resistance in Enterococcus is of alarming and great concern in IPD patients.

Urinary tract infections are predominantly caused by *Escherichia coli*, followed by *Klebsiella pneumoniae*. Trend of organism in both OPD and IPD patients is similar, except for higher percentage of infections by nonfermenters in IPD patients.

On comparing antimicrobial sensitivity of gram negative isolates, OPD isolates were more sensitive and the difference were statistically significant for antimicrobials like amikacin, nitrofurantoin, norfloxacin, meropenem, cefoperazone- sulbactam, piperacillin tazobactam and aztreonam.

In our study gram negative uropathogens had low susceptibility to ampicillin, cotrimoxazole, norfloxacin and cephalosporins. They had good susceptibility to nitrofurantoin and aminoglycosides like amikacin, gentamicin followed by piperacillin-tazobactam and meropenem. In our study 40% of the *E. coli* isolates and 60% of *Klebsiella spp* were Extended spectrum β lactamase (ESBL) producers.

4. 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 et al. [16] and Lakshmi et al. [1] showing significant bacteriuria as 26.01% and 23.85% respectively.

In our study amongst the gram negative bacteria, *Escherichia coli* was the predominant pathogen followed by *Klebsiella pneumoniae* and other Enterobacteriaceae. Similarly, amongst gram positive cocci, there was predominance of *Enterococcus* followed by *Staphylococcus aureus*. Global Prevalence of infections in urology, web-based multinational, multicentre point study carried in 70 countries showed similar trend of organisms with predominance of *Escherichia coli*, *Klebsiella pneumoniae,* followed by other enterobacteriaciae and *Pseudomonas aeruginosa* amongst gram negative bacilli. It also showed predominance of *Enterococcus* followed by *Staphylococcus aureus* amongst gram positive cocci [17]. This finding is consistent with other Indian studies as well [1,10,11,18,19]. 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 [18].

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 [19]. In another study from Rajasthan, Dalela etal reported 73% of *E. coli* and 59% of *Klebsiella species* from uropathogens to be ESBL producers [11]. 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% [11]. Aggarwal et al. also reported prevalance of MRSA in uropathogens as 36.84% [20]. 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 [16].

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). Similarly 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 and developed countries. [1,11,16,21]. 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 fluoroquinolones is also documented in other studies [1,16,21]. 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.

Global Prevalence of infections in urology, web-based multinational, multicentre point study carried in 70 countries across 4 continents Asia, Africa, Europe and America showed low sensitivity to cotrimoxazole, cephalosporins and fluoroquinolones [17].

In the present study, a good sensitivity to nitrofurantoin amongst gram positive isolates (OPD – 86% and IPD 83%) and gram-negative

Table 3. Percentage of multi drug resistant gram positive isolates

No.	Parameters	OPD (% of sensitivity)	IPD (% of sensitivity)
1	Percentage of MRSA	0 (0/5)	36% (4/11)
2	Percentage of HLAR Enterococcus	50% (9/18)	58% (78/135)
3	Percentage of VRE	0 (0/18)	2% (3/136)

Table 4. Distribution of gram negative isolates in UTI (n=814)

No	Name of isolate	OPD (n=121)	IPD (n= 693)	Total (n=693)
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 nonfermenter GNB*	3 (2%)	20 (3%)	23
8	*Proteus spp*	-	17 (2%)	17
9	Total	121	693	814

Table 5. Antimicrobial sensitivity testing of gram negative isolates (n=814)

No.	Antibiotic	OPD (% of sensitivity) (n=121)	IPD (% of sensitivity) (n=693)	P value P<0.001 – Statistically 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 sulbactam	83 (69%)	311 (45%)	P value =0.0001 (HS)
10	Piperacillin tazobactam	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)

isolates (87% in OPD and 65% in IPD patients) was observed. Our findings are like other Indian studies which have also demonstrated nitrofurantoin as an appropriate agent for first line treatment of community acquired UTIs [1,16,21]. 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. Gram negative isolates from OPD had sensitivity of 72% and 61% to amikacin and gentamicin respectively. Staphylococcus isolates from OPD setup also showed 100% sensitivity to gentamicin.

As per Global Prevalence of Infections in Urology worldwide surveillance study resistance rates of all antibiotics tested other than carbapenems against the total bacterial spectrum were higher than 10% in all regions. Resistance to almost all pathogens was lowest in North Europe and highest in Asia [2].

So, recommendations based on findings of our study in our set up are for uncomplicated non-hospitalised patient's nitrofurantoin is the best antimicrobial. For complicated Urinary tract infections or serious hospitalized patient's aminoglycosides, or β lactam-β lactamase inhibitor combination agents like piperacillin/ tazobactum and cefaperazone sulbactam can be effective. Carbapenems should be reserved for very serious hospital acquired infections.

5. CONCLUSION

Among the oral drugs norfloxacin, tetracycline 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. Parenteral drugs such as aminoglycosides, and Beta lactam and beta lactam inhibitor combination agents like piperacillin/tazobactam, cefaperazone-sulbactam can be the alternative for complicated UTI. Carbapenems should be reserved for very serious life-threatening infections. Escalation or de-escalation 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 MDR strains. It is essential to test and report ESBLs, Vancomycin resistance in enterococcus and MRSA production along with the routine susceptibility testing, which will help the clinicians in prescribing proper antibiotics.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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