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ORIGINAL ARTICLE

CHANGING PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PROFILES BY UROPATHOGENS: A STUDY IN INDIA

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ABSTRACT: AIM: To analyse the changing spectrum of uropathogens isolated from clean catch mid-stream urine samples and to evaluate the antibiotic sensitivity pattern & Multi drug resistance of those isolates. **MATERIALS AND METHODS:** All the urine samples collected in sterile container were received during the study period from Jan 2013 to Dec 2014 were processed & all the pathogenic isolates were identified as per the standard guideline. Antibiotic sensitivity was performed for the identified pathogens according to CLSI standards. **RESULTS:** A total 2306 urine specimens were processed and 43.06% showed significant bacteriuria among which 31.78% revealed GNB bacteriuria and 11.28% revealed GPC bacteriuria. The most frequently isolated pathogens were E.coli (27.8%), Klebsiella (16.7%) & Pseudomonas (6.0%) among GNB and Enterococci (11.9%) followed by CONS (8.6%) among GPC. Sensitivity tested against various antimicrobials to gram negative bacilli showed maximum sensitivity against Imipenem, Nitrofurantoin and Amikacin in order of sensitivity. Gram positive cocci showed maximum sensitivity against Vancomycin, Nitrofurantoin and Gentamicin respectively. Multidrug resistance was observed in all isolated pathogens. **CONCLUSION:** In this study we observed that there was a gradual shift in the prevalence of the isolated uropathogens and its antimicrobial sensitivity pattern. The reasons for this shift can be framed out as either more frequent and unnecessary usage of antibiotics or prescribing newer antibiotics with newer combinations for faster recovery of infections. Overall, both gram positive and gram negative organisms were most susceptible to Nitrofurantoin (Apart from Vancomycin in gram positives).
KEYWORDS: Antibiotic susceptibility**,** Escherichia coli, Urinary tract infections.

INTRODUCTION: Urinary Tract Infection [UTI] remains one of the most common bacterial infections and second most common infectious disease in the community practice. Approximately about 150 million people were diagnosed with UTI each year. [1] UTI may involve only the lower urinary tract or may involve both the upper and lower tract. The term cystitis has been used to describe lower UTI, which is characterized by a syndrome involving dysuria, frequency, urgency and occasionally suprapubic tenderness. However, the presence of symptoms of lower tract without upper tract symptoms does not exclude upper tract infection, which is also often present.[2] Inappropriate and empirical usage of wide spectrum antibiotics, insufficient hygiene, immunosuppression and prolonged hospitalization are some of the major aetiological factors that elevate the chances of infection.[3]

Among both outpatients and inpatients, Escherichia coli is the primary clinically prevalent organism, accounting for 75% to 90% of uncomplicated UTI isolates.[4] The treatment of UTIs varies according to the age of the patient, sex, underlying disease, infecting agent and whether there is lower or upper urinary tract involvement. In almost all cases, there is a need to initiate empirical antimicrobial treatment before obtaining the microbiological results.

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Surveillance studies provide information of the causative agents of UTIs and their antimicrobial resistance patterns which may aid clinicians in choosing the appropriate antimicrobial empirical treatment.[5] Knowledge on the antibiotic resistance patterns of the pathogens is important not only to provide an appropriate therapy, but also for the prevention of resistance amongst the microbes. If the treatment is given without considering the prevalent microbe and its antibiotic resistance pattern, it will results in the selection of more resistant strain.[6] In order to apply an appropriate therapeutic strategy, we must have data on the most common pathogens and also their sensitivity to different antibiotics.

There were many studies conducted in the past exposed the common uropathogens that caused UTI and also the gender that was more vulnerable. The commonest infecting organism and pattern of resistance keep changing over time. Hence, the present study was performed to analyze the changing etiologic trends of UTI and to evaluate the antibiotic sensitivity pattern & Multi drug resistance of those isolates.

MATERIALS AND METHOD:

Study Design: Retrospective & Prospective Study,

Study Period: Retrospective Study: January 2013 – December 2013, Prospective study: January 2014 – December 2014. The study was carried out after obtaining approval from Institutional Ethical Committee.

Retrospective Study: January 2013 to December 2013. Data were collected from microbiology culture register such as patient name, age, sex, ward, organism isolated and their antibiotic sensitivity pattern.

Prospective Study: All the clean catch mid-stream urine samples collected in sterile wide mouthed containers from inpatients of Annapoorana Medical College & Hospitals, Salem with suspected urinary tract infections, during the study period of January 2014 to December 2014 were included in this study. The urine thus collected were immediately transported to the diagnostic microbiology department and processed. In case of delay the samples were refrigerated at 4°C. The urine samples were subjected to various tests as follows.

Wet Mount: A drop of uncentrifuged well mixed urine was examined under the high power (40X) objective of the microscope for the presence of pus cells, RBCs, Bacteria, yeast, crystals/casts etc. Pyuria is>3 pus cells/HPF.[7]

Gram's Staining: Smear was prepared using a loopful of uncentrifuged well mixed urine. Air dried, heat fixed, Gram's stain was performed and observed under 100X (oil immersion field). The number of pus cells, epithelial cells and the Gram reaction & morphology of the organism, if present were recorded. The presence of one organism/Oil immersion field, correlates to 1, 00,000 colonies or more by culture.

Culture.[8]: A semi quantitative method was adopted for the primary isolation of the organism using a calibrated loop which delivers 0.01 ml of urine. A loopful of well mixed uncentrifuged urine was streaked on to the surface of Blood agar with 5% sheep blood and Mac Conkey agar. The culture plates were incubated for 24 hours at 37°C.

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After 24 hours of incubation, the number of colony forming units (CFUs) was multiplied by 100 to determine the number of microorganisms per millilitre in the original specimen. Cultures with colony counts >10^5/ml were considered as significant bacteriuria.

The organisms were identified using standard bacteriological techniques.[9] The species isolates were subjected to antibiotic susceptibility testing.

Antibiotics susceptibility testing.[10]: All the isolates thus speciated were subjected to antibiotic susceptibility testing by the Kirby Bauer's disc diffusion method. The list of Antimicrobial agents used in the study is given in the Table: 1. Appropriate antibiotics were tested for gram positive cocci and gram negative bacilli. Multi drug resistance such as Methicillin resistant Staphylococci aureus (MRSA) and Extended Spectrum of Beta Lactamase's (ESBL) were identified by standard phenotypic methods as per CLSI guidelines. In all ESBL producers the antibiotic susceptibility pattern to Imipenem was recorded by Kirby-Bauer method as per CLSI guidelines. Isolates were considered as resistant to Imipenem if the zone of inhibition was <13 mm, intermediate 14-15 mm and sensitive >16 mm.

Name of the group	Name of antimicrobials
Penicillins	Penicillin, Ampicillin, Piperacillin, Piperacillin/Tazobactam, Ampicillin with Sulbactam
Aminoglycosides	Gentamicin, Amikacin,
Cephalosporins	Cefazolin, Cefoxitin, Cephotaxime, Ceftriaxone, Ceftazidime
Fluoroquinolones	Norfloxacin, Ciprofloxacin, Ofloxacin
Glycopeptides	Vancomycin
Carbapenems	Imipenem
Oxazolidinone	Linezolid
Tetracycline	Tetracycline
Nitrofurans	Nitrofurantoin
Monobactams	Aztreonem
Polymyxins	Colistin
Others	Cotrimoxazole,

Table 1: List of Antimicrobial Agents used in the study

RESULTS: Of the total 2306 urine specimens processed during the study period (Jan 2013 to Dec 2014); 1045(45.3%) revealed culture negative whereas 993(43.1%) showed significant bacteriuria among which 733(31.8%) revealed GNB bacteriuria and 260(11.3%) revealed GPC bacteriuria (Table: 2). Candida species were isolated in 63(2.7%) samples and more than three agents (polymicrobial) were grown in 205(8.9%) samples.

Gram Negative Isolates	Gram Positive Isolates	Growth	No growth	Total
733	260	993	1045	2306
31.8%	11.3%	43.1%	45.3%	100.0%

Table 2: Patterns of Culture results

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The Demographic data such as Age, sex and ward distribution of bacterial isolates were noted. In our study UTI was common in the age group of 40-60 years (38%) followed by above 60 years (31%). It was observed that number of female patients was significantly higher (67.9%) than the number of male patients (32.1%) during both the years of study.

The male to female ratio was approximately 1:2. The distribution of the bacterial isolates from the Urine samples in the various wards were given in Table: 3.

Ward	Total number of isolates	Percentage	Gram Negative Isolates	Percentage	Gram Positive Isolates	Percentage
ICU	289	29%	222	30%	67	26%
MW	402	41%	342	47%	60	23%
OG	106	11%	66	9%	40	15%
PAED	104	10%	57	8%	47	18%
SW	81	8%	39	5%	42	16%
ORTHO	11	1%	7	1%	4	2%
TOTAL	**993**	**100%**	**733**	**100%**	**260**	**100.00%**

Table 3: Distribution of bacterial isolates from urine samples in various wards

Note: ICU-Intensive care unit, MW-Medical ward, OG-Obstetrics &Gynaecology, PAED-Paediatrics, SW-Surgical ward, ORTHO-Orthopaedics.

The commonest gram-negative bacilli isolated was E.coli (27.8%) followed by Klebsiella species (16.7%) during both the years. Among the gram-positive organisms, Coagulase Negative Staphylococcus (13.0%) was the most prevalent in 2013 and Enterococci (15%) in 2014. The other uropathogens encountered were Proteus species, Citrobacter species, Acinetobacter species, Staphylococci aureus and Candida species etc. The list of uropathogens isolated from the urine samples was shown in Table 4.

Sl. No.	Organism isolated	2013	2014	TOTAL	%
1	Escherichia coli	139	211	350	27.8%
2	Klebsiella pneumoniae	82	129	211	16.7%
3	Pseudomonas aeruginosa	43	33	76	6.0%
4	Citrobacter species	17	33	50	4.0%
5	Proteus species	9	22	31	2.5%
6	Acinetobacter baumanii	4	11	15	1.2%
7	Enterococcus species	39	111	150	11.9%
8	Coagulase Negative Staphylococci	65	44	109	8.6%
9	Staphylococcus aureus	1	0	1	0.1%
10	Candida species	35	28	63	5.0%
12	Total number of isolates	434	622	1056	83.8%

Table 4: common uropathogens

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Note: Polymicrobial aetiology was encountered in 205(16.2%) of the total isolates which were rejected from the study.

Table 5 to 7 shows antimicrobial sensitivity pattern of the most common three gram-negative isolates All the Gram-negative isolates showed high sensitivity to Amikacin, Nitrofurantoin & Imipenem. These isolates showed low sensitivity to all the commonly used antibiotics. The main challenge is the multidrug resistance (i.e. resistance to more than 3 drug) shown by all the isolates.

	GEN	AK	COT	NIT	NX	OF	CTR	CAZ	IPM
2013	41%	81%	19%	84%	16%	44%	7%	12%	100%
2014	29%	90%	29%	97%	24%	29%	42%	42%	100%
Overall %	34%	86%	25%	92%	21%	35%	28%	30%	100%

Table 5: Analysis on changing antibiotic sensitivity pattern of E.coli

Note: GEN- Gentamicin, AK- Amikacin, COT- Trimethoprim/Sulfamethoxazole, NIT- Nitrofurantoin, NX- Norfloxacin, OF- Ofloxacin, CTR- Ceftriaxone, CAZ- Ceftazidime, IMP-Imipenem, PI- Piperacillin, PIT- Piperacillin/Tazobactam, AT- Aztreonem

	GEN	AK	COT	NIT	NX	OF	CTR	CAZ	IPM
2013	63%	59%	32%	48%	22%	48%	22%	22%	89%
2014	30%	78%	30%	78%	27%	39%	32%	27%	100%
Overall%	43%	71%	31%	66%	24%	42%	26%	24^%	96%

Table 6: Analysis on changing antibiotic sensitivity pattern of Klebsiella spp

	GEN	AK	NX	OF	CAZ	IPM	PI	PIT	AT
2013	52%	21%	9%	61%	30%	100%	100%	100%	100%
2014	51%	18%	18%	67%	33%	100%	100%	100%	100%
Overall%	51%	20%	13%	63%	32%	100%	100%	100%	100%

Table 7: Analysis on changing antibiotic sensitivity pattern of Pseudomonas spp

Overall, 500 out of the 642 Gram negative isolates (78%) were presumptive ESBL producers, as detected by the combination disc method. Furthermore, 76% of E. coli, 79% of Klebsiella spp. and 90% of Citrobacter spp. isolates were ESBL producers. Carbapenam resistance was detected in 11% of Klebsiella spp. in 2013

The percentage sensitivity of Gram positive bacterial isolates to each antibiotic tested was shown in Table: 8. All the Gram positive bacteria were 100 percent susceptible to Vancomycin and Linezolid and least sensitive to Penicillin. Among the common antibiotics tested against all Gram positive bacteria, the most effective antibiotic was found to be Nitrofurantoin (79%) followed by Gentamicin (64%). Methicillin Resistant Coagulase Negative Staphylococci (MRCONS) isolates accounted for 61 out of 109 (56%).

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Antibiotics	Enterococcus species		Coagulase Negative Staphylococci	
	2013	2014	2013	2014
P	10%	15%	20%	44%
AMP	23%	20%	40%	53%
HLG/G	23%	30%	66%	64%
COT	NT	NT	34%	14%
NIT	23%	25%	74%	79%
NX	33%	35%	26%	50%
TC	33%	35%	NT	NT
VA	100%	100%	100%	100%
LZ	100%	100%	100%	100%

Table 8: Antimicrobial sensitivity profile of Gram positive isolates

Note: P-Penicillin, AMP-Ampicillin, HLG-High Level Gentamicin, GEN- Gentamicin, COT-Cotrimoxazole, NIT- Nitrofurantoin, NX- Norfloxacin, TC-Tetracycline, VA-Vancomycin, LZ-Linezolid

DISCUSSION: Urinary tract infections are the most frequent clinical manifestation after respiratory tract infections. It was observed that numbers of female patients were significantly higher than the number of male during both the years of study. The male to female ratio was approximately 1:2 and this ratio corroborates with the previous reports.[11] i.e. UTI is more prevalent in females. In our study gram negative organisms (31.8%) were predominant over the gram positive ones (11.3%) as shown in Table: 2. When we analyzed the frequency of distribution of the uropathogens, 41% of the cases were from medical ward, 29% from ICU, 11% from O&G and 10% from paediatrics (Table: 3). UTI cases from ICU would have had infection earlier or due to previous catheterisation because in all cases urine sample was collected within 48 hours of admission.

UTIs are caused by a variety of microorganisms, including both gram negative and gram positive ones. In our study Escherichia coli (27.8%) was predominant isolate followed by Klebsiella spp. (16.7%) and Ps. aeruginosa (6.0%) respectively. This finding agrees with other reports which indicated that gram negative bacteria mostly E. coli & Kleb. Pneumonia are the commonest pathogens isolated in patient with urinary tract infections.[12, 13, 14, 15] However there was marked increase in the UTI cases due to Proteus species during 2014 was noticed, thereby suggesting hospital acquired infection. Among the gram positive bacteria there is a change in microbial spectrum during the study period as shown in Table: 4. CONS were the most prevalent one during 2013 at 13% followed by Enterococci at7.5%. This profile altered during 2014, where in Enterococci prevalence rate increased to 15% followed by CONS at 5.9%.

We reviewed not only the changing bacterial profile but also their sensitivity pattern during the study period. Similar to our study the changing antibiotic sensitivity pattern was also analyzed and discussed in a study by Ram S et al.[11] Analysis on changing sensitivity pattern of the three most prevalent gram negative uropathogens E. coli, Klebsiella spp & Pseudomonas spp at our tertiary care centre is shown in Table:5, Table: 6 and Table: 7 respectively.

Analysis on antibiotic sensitivity pattern of E. coli & Klebsiella spp shows that there was relatively overall good sensitivity percentage for Amikacin (86 & 71%), Nitrofurantoin (92 & 66%) and Imipenem (100 & 96%) respectively. This is in accordance with the study by Suneel Bhooshan

et al.[16] Imipenem sensitivity was 100% in E. coli in both years but for Klebsiella it was only 89% in 2013 and 100% in 2014. All the isolates which shows low sensitivity to Imipenem were ESBL producing Klebsiella from ICU. Low sensitivity of Klebsiella to Imipenem was shown by Manikandan C et al. in 2013 (86.1%), [12] A Khorshidi et al in 2010 (87.5%).[17] and Farhat Ullah et al in 2009 (86.96%).[18] There was an increase in percentage of sensitivity for Norfloxacin, Ceftriaxone and Ceftazidime in 2014 when compared to 2013 but the sensitivity towards Gentamicin & Ofloxacin decreased in 2014. In our study the range of sensitivity for Norfloxacin, Ceftriaxone, Ceftazidime were 20 percent to 30 percent and for Gentamicin & Ofloxacin 30% to 40%.

This emerging resistance towards these antibiotics may be attributed to the indiscriminate use of these antibiotics by the general practioners. The sensitivity of Ampicillin was 0% and Co-trimoxazole was 25% to 31%.

Studies that were conducted in India showed that the isolates of E.coli & Klebsiella showed high resistance towards Ampicillin and Co-trimoxazole which was in agreement with our study.[1, 4, 19,20] Multi drug resistance to E.coli & Klebsiella at our hospital setting were at 79% & 91% and ESBL producing E.coli & Klebsiella were 76% & 79% respectively. In a study by Gupta N, et al.[5] MDR to most of the uropathogens were 100 percent. This rapidly rising prevalence of ESBL production among Enterobacteriaceae is the result of selection pressure due to massive prescription and often misuse of broad-spectrum antibiotics, including Cephalosporins, both in hospitals and the community.

This multidrug resistance is due to simultaneous transmission of ESBL gene with plasmids that encode resistance to Aminoglycosides, Quinolones and Trimethoprim/Sulfmethoxazole.[21] Among the urinary ESBL-producing K. pneumoniae isolates (n=60), nine strains (11%) showed a cross-resistance to carbapenems, which indicates an emergence of Carbapenem-resistant strains of K. pneumoniae. In various studies, across the world varying resistance (4-60%) has been seen towards this drug by Klebsiella spp isolated from various samples. In a study by M.C. El Bouamri et al.[22] in 2015, Carbapenem - resistance by urinary isolates was 7%.

Third most Prevalent pathogen Pseudomonas had overall very good sensitivity to Gentamicin at 51% & Ofloxacin at 63%. Imipenem, Piperacillin, Piperacillin/Tazobactam, Aztreonem & Colistin sensitivity were consistently at 100% throughout the study period. The other antibiotics which showed decreased sensitivity percentage were Amikacin (20%), Norfloxacin (13%) and Ceftazidime (32%). MDR percentage of Pseudomonas at our centre was 51%.

In our study, Vancomycin was found to be the most effective drug against gram positive bacteria. However, in CONS (most prevalent in2013) the sensitivity to Nitrofurantoin (79%) and Gentamicin (65%) were found to be high which is similar various studies done previously. Penicillin (44%), Ampicillin (53%) and Norfloxacin (55%) showed moderate sensitivity and Co-trimoxazole (14%) sensitivity was low. Enterococcus (most prevalent in 2014) was found to have low sensitivity to all antibiotics tested which ranges from 20% to 35%.

Low sensitivity could be due to MDR which was 67% in our study. Multi drug resistance in urinary isolates of Enterococcus in various Indian studies ranges from 55.5% to 78.8%.[23, 24]

CONCLUSION: Various studies on antibiotic resistance are emerging with an intention to bring into lime light about the resistance that is developing among the microorganisms towards the antibiotics. Though the approaches are same, the results are showing entirely different conclusions from time to time. However, reasons for the cause of this resistant activity can be framed out as either more

frequent and unnecessary usage of antibiotics or prescribing newer antibiotics with newer combinations for faster recovery of infections. Overall, both gram positive and gram negative organisms were most susceptible to Nitrofurantoin (apart from Vancomycin in gram positives) possibly because it is not used for other infections and shows good in vitro activity. Thus, on the basis of our results it may be considered as first line empirical oral therapy for ambulatory patients. Use of drugs such as Amikacin, Imipenem and Piperacillin-Tazobactam is emphasized for treatment of gram negative UTI. Inappropriate usage of antimicrobials in surgical perioperative prophylaxis should be prohibited and a close collaboration between physicians, surgeons and microbiologists established. Last but not the least; continued evaluation of susceptibility pattern of uropathogens to traditional as well as new antimicrobials in well-defined populations should be done to ascertain the optimal empiric therapy.

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