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UROPATHOGENS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL

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ABSTRACT: BACKGROUND: Urinary tract infections (UTI) are one of the most common infectious diseases in humans. Antimicrobial drug resistance is one of the major threats due to wide spread use of inappropriate and empirical antibiotic therapy. The present study highlights the organisms causing UTI and their antimicrobial susceptibility pattern. **MATERIALS AND METHODS:** A total number of 1266 urine samples were analysed in a tertiary care hospital. Urine samples were processed by calibrated loop technique, delivering 0.001 ml of urine and plated on to MacConkey's and Cysteine Lactose Electrolyte Deficient (CLED) agar plates and incubated at 37^0C overnight. Appropriate biochemical tests were carried out to identify the uropathogens. Antimicrobial susceptibility of the isolates was determined against various antimicrobial agents. **RESULTS:** Among this, 332(26.22%) culture positive isolates were identified and their antimicrobial susceptibility patterns were analysed. Among the culture positive isolates, Escherichia coli (54.21%)was the most common pathogen followed by Klebsiella spp (22.89%) and Enterococcus spp (6.92%). Majority of these isolates were resistant to Cotrimoxazole, Amoxycillin/ Clavulanic acid, Piperacillin, Norfloxacin, Ciprofloxacin and also to third generation cephalosporins. Escherichia coli isolates showed good sensitivity towards Amikacin, Piperacillin/ Tazobactum, Nitrofurantoin and Meropenem. Klebsiella isolates showed good sensitivity towards Amikacin, Levofloxacin, Piperacillin/ Tazobactum and Meropenem. Staphylococcus aureus and Enterococcus spp showed good response towards Linezolid, Netilmicin, Vancomycin and Teicoplanin. There is also increase in incidence of Extended Spectrum Beta Lactamase (ESBL) production among Escherichia coli (65%) and Klebsiella spp (55%). Study also showed that females (67.17%) are more vulnerable to urinary tract infections than males (32.83%). **CONCLUSION:** Due to excessive use of antimicrobials for all sorts of infections, uropathogens are increasingly showing resistance to antibiotics. Knowledge of uropathogens and their antimicrobial susceptibility pattern in a geographical region will help in appropriate and judicious antibiotic usage in a health care setup.
KEYWORDS: Urinary Tract Infection, Antibiotic Susceptibility Pattern, ESBL

INTRODUCTION: Urinary tract infections (UTI) are one of the most common infectious diseases in humans. Antimicrobial drug resistance is one of the major global threats.[1]Antibiotics are usually given empirically before the laboratory reports of urine cultures are available.

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Inappropriate and empirical usage of wide spectrum of antibiotics, insufficient hygiene, immunosuppression and prolonged hospitalisation are some of the major etiological factors that increase the chances of UTIs.[2]Wide spectrum of organisms are implicated in its aetiology, the most common being Escherichia coli and Klebsiella spp followed by Gram positive organisms.[3]

Gram negative bacilli shows high incidence of resistance against Cotrimoxazole, Norfloxacin, Piperacillin, Fluoroquinolones and third generation cephalosporins.[4,5]Gram positive cocci shows high incidence of resistance against Ampicillin, Amoxycillin, Penicillin, Amoxycillin/ Clavulanic acid and Erythromycin. Antibiotic susceptibility pattern of uropathogens vary according to the geographical and regional locations. The knowledge about uropathogens and their resistance patterns is important for appropriate therapy and also for prevention of resistance amongst the microbes. In this context present study was carried out to know the common uropathogens and their susceptibility pattern in a tertiary care hospital.

MATERIALS AND METHODS: A total of 1266 clean catch midstream urine samples were collected in a sterile container from both outpatients and inpatients in our hospital during study period (January 2010 – December 2011). Urine samples were transported immediately to microbiology laboratory and processed. Urine samples were processed by calibrated loop technique delivering 0.001 ml of urine and plated on to MacConkey's and Cysteine Lactose Electrolyte Deficient (CLED) agar plates and incubated at 37^0C overnight. For Gram negative bacilli more than 10^5 colonies per ml and for Gram positive cocci 10^3-10^5 colonies per ml of single organism were considered significant. Uropathogens were further identified by the morphological and biochemical characteristics.[6]

Antimicrobial susceptibility of the isolates was determined against various antimicrobial agents by Kirby Bauer disk diffusion method on Muller Hinton agar plates according to Clinical and Laboratory Standard Institute(CLSI) guidelines.[7]Antibiotics for Gram negative bacilli included Amikacin(AK-30µg), Ceftazidime (Ca- 30 µg), Cefotaxime (Ce-30µg), Ceftriaxone (Ci-30 µg), Cefepime (Cpm- 30µg), Ciprofloxacin (Cf- 5µg), Cotrimoxazole (Co-25µg), Gentamycin (G -10µg), Levofloxacin(Le-5µg), Piperacillin(Pc-100µg), Piperacillin- Tazobactum(Pt- 100/10µg), Norfloxacin (Nx -10µg), Nitrofurantoin (Nf - 300µg), Ofloxacin (Of - 5µg), Meropenem (Mr-10µg), Aztreonam (Ao-30µg). Escherichia coli and Klebsiella isolates were tested for Extended Spectrum Beta Lactamase (ESBL) production by double disk diffusion method with Ceftazidime and Ceftazidime Clavulanic acid combination as recommended by CLSI guidelines.[7, 8]Escherichia coli ATCC 25922 and Klebsiella pneumoniae ATCC 700603 strains were used as controls.

Gram positive cocci were tested for Ampicillin (A-10µg), Amoxycillin (Am -30 µg), Cephoxitin (Cn-30µg), Clindamycin (Cd-2µg), Erythromycin (E-15µg), Linezolid (Lz -30µg), Netilmicin (Nt -30µg), Penicillin (P-10 units), Teicoplanin (Te-30µg), Vancomycin (Va-30 µg) (Hi Media, Mumbai). Staphylococcus aureus ATCC 25923 and Enterococcus faecalis ATCC 29212 strains were used as controls. The results were recorded and interpreted according to CLSI guidelines.

RESULTS: Out of 1266 urine samples, 332(26.22%) were found to be culture positive isolates. Among 332 culture positive isolates, 223 (67.17%) were obtained from females and 109(32.83%) were obtained from males as shown in Table1. The highest isolation rates was found in 20 – 69 age group as shown in Table 2.

Escherichia coli(54.21%) was the predominant uropathogen, followed by Klebsiella spp(22.89%), Enterococcus spp(6.92%), Staphylococcus aureus (5.21%), Acinetobacter spp (3.31%) and Citrobacter spp (3.01%) as shown in Table 3. The antibiotic resistance among uropathogens to the agents that had been recommended as the first line therapy is on rise. The Gram negative bacilli which were isolated showed high degree resistance pattern against Cotrimoxazole, Norfloxacin, Piperacillin, Ciprofloxacin and third generation cephalosporins. Escherichia coli isolates showed higher sensitivity towards Amikacin (91.18%), Nitrofurantoin (90.52%), Piperacillin- Tazobactum (96.44%) and Meropenem (100%). Klebsiella isolates showed good response towards Amikacin (92.61%), Levofloxacin (90%), Piperacillin-Tazobactum (95%), Meropenem (100%) as shown in Table 4. ESBL production among Escherichia coli isolates were 65% and Klebsiella isolates, 55%. Enterococcus spp showed good response towards Linezolid (100%), Vancomycin (100%), and Teicoplanin (87.5%). Staphylococcus aureus showed good response towards Linezolid (88.1%), Netilmicin (87.5%), Vancomycin (100%), Teicoplanin (100%), as shown in Table 5.

DISCUSSION: The changing trends in the aetiopathogenesis of urinary tract infections and increasing antimicrobial drug resistance are a matter of concern. Urethral catheterisation and instrumentation related UTI is the most common nosocomial infection.[9] Catheter related UTI increases morbidity, mortality and also cost of treatment for patients in a health care setup.[10] The indiscriminate, inadequate usage of antibiotics has contributed to the emergence of resistance strains.[11]Urine culture sensitivity is routinely done in suspected cases of UTI and empirical therapy should be started immediately and modified if required once the report of urine culture sensitivity is available.[12, 13]

The present study shows the pathogens causing UTIs and their antibiotic susceptibility pattern. Escherichia coli was the predominant pathogen followed by Klebsiella spp and Gram positive cocci. This study is in concordance with the studies done by Manjunath et al,[1] and Shigemura et al. [7] Our study shows that females (67.17%) are more vulnerable to UTIs than males (32.83%), which is similar to previous studies done by Manjunath et al,[1]and Dash et al.[14] Females are more prone to UTIs probably due to their short urethra and physiological changes. Antimicrobial resistance of different uropathogens is one of the barricades that can interfere with an effective treatment. The present study indicates different antimicrobial susceptibility pattern among uropathogens. The broad spectrum activity of Fluoroquinolones and third generation Cephalosporin has made them therapeutic options for UTIs. The organisms which belonged to Enterobacteriaceae family, showed high degree of resistance towards Cotrimoxazole, Fluoroquinolones and third generation Cephalosporin, but these isolates showed good response against antibiotics like Amikacin, Nitrofurantoin, Piperacillin Tazobactum and Meropenem. This finding is similar to the studies done by Manjunath et al,[1]and Shigemura et al.[7] Our study also shows increase in ESBL producing strains among Escherichia coli (65%) and Klebsiella spp (55%), which is similar to the results of the studies done by Shiju et al,[8]and Shukla et al.[15] ESBL producing organisms pose a major problem in clinical therapeutics, so there is a need for judicious use of antimicrobial agents and their continuous Invitro monitoring should be carried out, so that misuse of extended spectrum cephalosporins can be avoided. Gram positive cocci showed good susceptibility patterns towards Netilmicin, Linezolid, Vancomycin and Teicoplanin.

This study furnishes the much needed information on the common uropathogens and their antibiotic susceptibility pattern in our region. In view of the emerging drug resistance

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among uropathogens, therapy should be commenced after the culture and sensitivity has been performed. This would, not only help in the proper use of antibiotics but also prevent further development of bacterial drug resistance in community as well as in hospitals.

Table 1: Sex- wise distribution of UTI

GENDER	Number of Isolates
Male	109(32.83%)
Female	223(67.17%)

Table 2: Age-wise distributions of uropathogens

Age group	Male	Female	Total
0-9	3	18	21
10-19	10	35	45
20-29	15	58	73
30-39	16	36	52
40-49	23	23	46
50-59	13	23	36
60-69	23	20	43
70-79	3	8	11
80-89	3	2	5

Table 3: Clinical strains isolated from urine samples.

Uropathogens	No. of isolates	Percentage
Escherichia coli	180	54.21%
Klebsiella spp	76	22.89%
Acinetobacter spp	11	3.31%
Citrobacter spp	10	3.01%
Pseudomonas spp	8	2.40%
Provedencia spp	4	1.20%
Proteus mirabilis	2	0.60%
Enterobacter spp	1	0.30%
`Enterococcus spp	23	6.92%
Staphylococcus aureus	17	5.12%

Table 4: Antibiotic Susceptibility pattern(% sensitivity): Gram negative bacilli.

Uropathogen	Ak	Ao	Ca	Ce	Ci	Cpm	Cf	Co	G	Le	Mr	Nx	Nf	Of	Pc	Pt
E.coli	91.18	ND	36.99	33.38	32.34	35.88	31.20	28.31	58.67	60.94	100	23.77	90.52	53.47	23.74	96.44
Klebsiella Spp	92.61	ND	46.89	39.64	41.3	49.51	53.75	49.61	68.35	90	100	50.69	70	43.18	29.11	95
Acinetobacter spp	83.33	ND	71.42	66.66	66.66	ND	66.66	50	70	100	81.81	75	ND	50	50	81.81
Citrobacter Spp	79.16	ND	65	45	66.66	40	79.16	55	73.33	75	100	60	70.8	70	40	87.5
Proteus Spp	100	ND	50	50	50	50	50	50	ND	100	100	50	100	50	ND	100
Pseudomonas Spp	50	37.5	50	50	37.5	ND	37.5	37.5	ND	50	75	50	ND	37.5	62.5	87.5
Providencia Spp	100	ND	50	50	50	ND	50	25	ND	75	100	50	75	50	50	100
Enterobacter spp	100	ND	-	-	-	ND	-	-	ND	100	100	-	100	-	-	100

Table5: Antibiotic Susceptibility pattern(% sensitivity): Gram positive cocci

	A	Am	Ac	Cd	Cz	Cn	E	G	Lz	Nt	Nx	Nf	P	Va	Te
S.aureus	25	29.16	50	70.1	53.5	53.57	52.94	63.85	88.1	87.5	ND	ND	11.8	100	100
Enterococcus Spp.	14.28	20	36	ND	ND	ND	36	46.66	100	ND	40	60	20	100	87.5

*ND-NOT DONE.

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