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A study of bacteriological and antibiotic susceptibility profile of urinary tract infection

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Abstract

Introduction: Urinary tract infection (UTI) is one of the most common infection and is associated with significant morbidity in the community. Most of the UTI cases are treated empirically with broad-spectrum antibiotics which invariably results in the development of resistance. **Aims and Objectives:** The objective of this study was to determine the antibiotic susceptibility pattern of bacterial isolates causing UTI and to determine Extended spectrum beta Lactamase (ESBL) production in Gram negative isolates. **Materials and Methods:** A total of 724 urine samples were studied and bacteria identified by standard microbiological methods. Antibiotic sensitivity pattern was done by Kirby-Bauer disc diffusion method. Detection of ESBL was done as per Clinical and Laboratory Standards Institute (CLSI) guidelines. **Results:** Significant bacteriuria was detected in 238 (32.8%) samples. The most common pathogens isolated were *Escherichia coli* 148 (58.9%), *Klebsiella pneumoniae* 57 (22.7%) and *Staphylococcus aureus* 18 (7.1%) followed by *Enterococcus spp* 7 (2.7%), *Proteus mirabilis* 6 (2.4%), *Citrobacter koseri* 6 (2.4%), *Pseudomonas aeruginosa* 5(2%) and *Staphylococcus saphrophyticus* 4 (1.6%). ESBL production was seen in *Klebsiella pneumoniae* 12 (21%), followed by *Escherichia coli* 26 (17.5%). Most of the Gram-negative bacteria were susceptible to meropenam, piperacillintazobactum and nitrofurantoin. **Conclusion**: This study reveals that many bacteria causing UTI are multidrug resistant pathogens. This suggests that regular monitoring and modification of empirical therapy and it's validation by culture report is required to prevent morbidity associated with this disease.

Keywords: Urinary tract infection, Extended spectrum beta lactamase, Drug resistance.

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Introduction

Urinary tract infections (UTI) is the most common bacterial infections that lead to seek medical care. About 150 million people develop UTI each year globally [1]. UTI are also the most common hospitalacquired infections, accounting for as many as 40% of nosocomial infections [2]. The problem of UTI involve both males and females of all age groups including neonates. Malnutrition, low socio-economic status with poor hygiene, structural and functional abnormalities of urinary tract are few of the main predisposing factors UTI [3]. Escherichia coli, causing Klebsiella pneumoniae, Staphylococcus aureus, Coagulase negative staphylococci, Proteus mirabilis, Pseudomonas aeruginosa are the most common pathogenic microorganisms isolated in urine [4]. Most of the UTI cases are treated empirically with broad spectrum

Manuscript received: 8th August 2018 Reviewed: 18th August 2018 Author Corrected: 24th August 2018 Accepted for Publication: 27th August 2018 antibiotics without the use of culture and sensitivity testing to guide therapy. Treatment becomes even more challenging in the presence of risk factors such as higher age, co-morbid conditions like diabetes mellitus, renal stones and immunosuppression [5]. Extensive and inappropriate use of antibiotics has invariably resulted in the development of antibiotic resistance which has become a major problem worldwide [6].

Extended spectrum beta-lactamase (ESBL) are the results of mutations in the ubiquitous class A TEM or SHV beta-lactamases. TEM-1 accounts for the majority of beta-lactamase-mediated resistance. These are mainly produced by *Escherichia coli* and *Klebsiella* [7].

ESBL producing bacteria show resistance not only to penicillins, cephalosporins and aztreonam but also to other classes of antibiotics such as aminoglycosides, cotrimoxazole, tetracycline and fluoroquinolones [8]. This increasing antimicrobial resistance complicates an uncomplicated UTI treatment by increasing patient morbidity, prolonged hospital stay, retreatment and use of broader spectrum of antibiotics.

Knowledge of the antimicrobial resistance pattern of common uropathogens according to local epidemiology is essential for providing clinically appropriate and costeffective therapy for UTI. Thus, this study was carried out to determine the prevalent uropathogens and antibiotic resistance patterns in our hospital.

Materials and Methods

Place of study: This study was carried out in the department of Microbiology at Sri Siddhartha Medical College, Hospital & Research Centre, Tumkur, during the period of January 2016 to December 2016.

Type of study: Prospective study **Inclusion criteria:** Clinically suspected cases of UTI

Exclusion criteria: Patients on antibiotics in prior week were excluded from the study.

Sample collection and method: A total of 724 consecutive, nonrepetitive urine samples were included in this study. A loopful (0.001 ml) of well mixed uncentrifuged urine was inoculated onto blood agar, Mac Conkey's agar and cysteine-lactose electrolyte deficient (CLED) agar.

All plates were then incubated at 37° C for 24 hrs. Significant growth was considered if colony count $\geq 10^{5}$ colony forming unit /ml (CFU/ml) based on Kass concept.

All the isolates were identified biochemically by the standard microbiological methods [9, 10].

Antimicrobial susceptibility testing: This was done on Muller Hinton agar by Kirby Bauer disc diffusion method according to the CLSI guidelines (11).

Results

Out of 724 urine samples, significant bacteriuria was seen in 238 (32.8%) samples which yielded 251 isolates.

The prevalence of UTI was higher in females (66.8%) and prevalence among males was (33.2%). 74 (10.2%) patients had an insignificant colony count.

No growth was seen in 345 (47.6%) specimens and mixed insignificant growth was seen in 67 (9.25%) samples.

The most common isolates were *Escherichia coli* 148 (58.9%), *Klebsiella pneumoniae* 57 (22.7%), *Staphylococcus aureus* 18 (7.1%) followed by *Enterococcus spp* 7 (2.7%), *Proteus mirabilis* 6 (2.4%), *Citrobacter koseri* 6 (2.4%), *Pseudomonas aeruginosa* 5(2%) and *Staphylococcus saphrophyticus* 4(1.6%).

All Enterobacteriaceae members were tested against ampicillin/ sulbactum $(10\mu g/10\mu g)$, nitrofurantoin $(300\mu g)$, amikacin $(30\mu g)$, gentamicin $(10\mu g)$, cefotaxime $(30\mu g)$, ceftriaxone $(30\mu g)$, ceftazidime $(30\mu g)$, cotrimoxazole $(1.25/23.75\mu g)$, ofloxacin $(10\mu g)$, piperacillin- tazobactum $(100/10\mu g)$ and meropenem $(10\mu g)$.

Staphylococci were tested against ampicillin/ sulbactum $(10\mu g/10\mu g)$, amikacin $(30\mu g)$, gentamicin $(10\mu g)$, ceftriaxone $(30\mu g)$, ciprofloxacin $(10\mu g)$, nitrofurantoin $(300\mu g)$, cotrimoxazole $(1.25/23.75\mu g)$, vancomycin $(30\mu g)$, linezolid $(30\mu g)$ and cefoxitin $(30\mu g)$.

Enterococcus spp were tested against amikacin $(30\mu g)$, high level gentamicin $(120\mu g)$ ceftriaxone $(30\mu g)$ vancomycin $(30\mu g)$,ciprofloxacin $(10\mu g)$, nitrofurantoin $(300\mu g)$ and linezolid $(30\mu g)$.

Test for Detection of ESBL Production in Enterobacteriaceae: Isolates which were resistant to third generation cephalosporins were tested for ESBL production by combination disk method using cefotaxime ($30\mu g$), cefotaxime/ clavulanic acid ($30\mu g/10\mu g$), and ceftazidime ($30\mu g$), ceftazidime/ clavulanic acid ($30\mu g/10\mu g$).

Plates were incubated overnight at 37° C. Zone of inhibition of ≥ 5 mm around cephalosporin + clavulanate compared to cephalosporin alone confirms ESBL production [11].

Test for detection of Methicillin resistance in Staphylococcus: The test was carried out on Muller-Hinton agar using a cefoxitin disc $(30\mu g)$ and incubated at 35°C for 18-24 hrs.

An inhibition zone diameter of ≤ 21 mm was reported as methicillin resistant and a diameter of ≥ 22 mm was reported as methicillin sensitive strains [11].

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SI.	Isolates	Amp/sul	G	Ak	Of	Со	Nit	Ca	Ce	Ctr	Pt	М
No.		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1	Escherichia coli	32	98	101	93	77	139	92	88	97	140	148
	(148)	(21.6)	(66.2)	(68.2)	(62.8)	(52)	(93.2)	(62.1)	(59.4)	(65.5)	(94.5)	(100)
2	Klebsiella	12	34	36	31	26	53	34	32	37	52	57
	pneumonia (57)	(21)	(59.6)	(63.1)	(54.3)	(45.6)	(92.9)	(59.6)	(56.1)	(64.9)	(91.2)	(100)
3	Proteus mirabilis	1	2	2	2	3	4	6	6	6	6	6
	(6)	(16.6)	(33.3)	(33.3)	(33.3)	(50)	(66.6)	(100)	(100)	(100)	(100)	(100)
4	Citrobacter koseri	2	4	4	5	3	5	5	5	6	5	6
	(6)	(33.3)	(66.6)	(66.6)	(83.3)	(50)	(83.3)	(83.3)	(83.3)	(100)	(83.3)	(100)
5	Pseudomonas	0	2	3	3	1	2	3	2	2	4	5
	aeruginosa (5)	(0)	(40)	(60)	(60)	(20)	(40)	(60)	(40)	(40)	(80)	(100)
	Total (222)	47	140	146	134	110	203	140	133	148	207	222

Table-1: Antibiotic sensitivity pattern of Gram negative organisms.

The predominant isolate *Escherichia coli* showed maximum sensitivity towards meropenem (100%), piperacillintazobactum (94.5%), nitrofurantoin (93.2%) and they were least sensitive towards ampicillin/ sulbactum (21.6%), ofloxacin (37.5%) and cotrimoxazole (52%). *Klebsiella pneumoniae* 57 (23.17%) was the second most common isolated organism and it was most sensitivite to meropenem (100%), nitrofurantoin (92.9%) and piperacillin-tazobactum (91.2%) and least sensitive to ampicillin/sulbactum (21%), cotrimoxazole (45.6%) and ofloxacin (54.3%) (Table-1).

Table-2: Antibiotic sensitivity pattern of Escherichia coli.

SI.	ESBL/	Amp/sul	G	Ak	Of	Со	Nit	Ca	Ce	Ctr	Pt	Μ
No.	Non-ESBL	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1	ESBL	0	12	10	12	11	22	0	0	0	22	26
	Escherichia coli-(26)	(0)	(46.1)	(38.4)	(46.1)	(42.3)	(84.6)	(0)	(0)	(0)	(84.6)	(100)
2	Non ESBL	32	86	91	81	66	117	92	88	97	118	122
	Escherichia coli(122)	(26.2)	(70.4)	(74.5)	(66.3)	(54)	(95.9)	(75.4)	(72.1)	(79.5)	(96.7)	(100)
	Total 148	32	98	101	93	77	139	92	88	97	140	148

Table-3: Antibiotic sensitivity pattern of Klebsiella pneumoniae.

SI.	ESBL/	Amp/sul	G	Ak	Of	Со	Nit	Ca	Ce	Ctr	Pt	Μ
No.	Non-ESBL	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1	ESBL Klebsiella	0	4	4	3	0	10	0	0	0	10	12
	pneumoniae (12)	(0)	(33.3)	(33.3)	(25)	(0)	(83.3)	(0)	(0)	(0)	(83.3)	(100)
2	Non ESBL	12	30	32	28	26	43	34	32	37	42	45
	Klebsiella pneumoniae (45)	(26.6)	(66.6)	(71.1)	(62.2)	(57.7)	(95.5)	(75.5)	(71.1)	(82.2)	(93.3)	(100)
	Total 57	12	34	36	31	26	53	34	32	37	52	57

Amp/sul-Ampicillin/ sulbactum, G-Gentamicin, Ak-Amikacin, Of-Ofloxacin, Co-Cotrimoxazole, Nit-nitrofurantoin, Ca-Ceftazidime, Ce-Ceftotaxime, Ctr-ceftriaxone, Pt-Piperacillin/ tazobactam, M-meropenem,

Among the 217 Enterobacteriaceae isolates, 64 (29.4%) were showing multidrug resistance (MDR). Among these 64 MDR isolates, 38 (59%) were ESBL producers. Highest prevalence of ESBL production was seen in *Klebsiella pneumoniae* 12 (21%), followed by *Escherichia coli* 26 (17.5%). The ESBL producing strains showed maximum sensitivity towards meropenem, nitrofurantoin and piperacillin-tazobactum and 100% resistance towards third generation cephalosporins and ampicillin/ sulbactum (Table-2, 3).

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SI.	Organisms	Amp/sul	G/HLG	Ak	Ctr	Cf	Co	Nit	Lz	Cn	Va
No.		(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1	Staphylococcus	2	13	12	14	6	8	16	18	16	18
	aureus (18)	(11.1)	(72.2)	(66.6)	(77.7)	(33.3)	(44.4)	(88.8)	(100)	(88.8)	(100)
2	Enterococcus	0	4	3	2	3	-	4	7	-	7
	species (7)	(0)	(57)	(42)	(28)	(42)		(57)	(100)		(100)
3	Staphylococcus	0	2	2	2	3	2	4	4	4	4
	saphrophyticus (4)	(0)	(50)	(50)	(50)	(60)	(50)	(100)	(100)	(100)	(100)
	Total-29	2	19	17	18	12	10	24	29	20	29

Table- 4: Antibiotic sensitivity pattern of Gram positive organisms.

Amp/sul-Ampicillin/sulbactum, G-Gentamicin, HLG-high level Gentamicin, Ak-Amikacin, Ctr-ceftriaxone, Cf-Ciprofloxacin, Co-Cotrimoxazole, Nit-nitrofurantoin, Lz-linezolid, Cn-cefoxitin, Va-vancomycin.

All the isolates of *Staphylococcus aureus* were sensitive to vancomycin and linezolid (Table-4). Among the *Staphylococcus aureus*, 2 (11.1%) were found to be MRSA by disc diffusion test. 50% of MRSA strains were sensitive to amikacin, gentamicin, ciprofloxacin and nitrofurantoin. They were 100% resistant to ampicillin/sulbactum, ceftriaxone and cotrimoxazole.

Discussion

For the appropriate empirical therapy of UTI, knowledge about present trends of the uropathogens and their susceptibility to various antibiotics is essential because studies have shown changing trends of susceptibility pattern from different places over a period of time [12, 13]. Our study showed a high prevalence of UTI in females (66.8%) than in males (33.2%) which correlates with other findings done by Orrett et al, Sood et al [14,15]. UTI is more common in females because of shorter urethra and urethra is more proximal to anus so that coliforms enter and colonize urethra [12, 15].

Out of 724 urine samples, 238 (32.8%) were found to be culture positive which yielded 251 isolates. *Escherichia coli* 148 (58.9%) was the predominant uropathogen which is in concordance with the other studies [2, 16]. Enterobacteriaceae have several factors responsible for their attachment to the uroepithelium. The Gram negative bacteria colonize the uroepithelium mucosa with adhesions, pili, fimbriae and P1 blood group phenotype [12, 17]. *Klebsiella pneumoniae* was the second most common organism isolated. Others have found an increase in *Klebsiella pneumoniae* causing UTI [18]. This increased trend may be due to increased colonization of multidrug resistant *Klebsiella pneumoniae* in hospital setup [15, 19].

The Gram-negative bacteria were showing maximum sensitivity towards meropenem and piperacillintazobactam followed by nitrofurantoin (91.4%), amikacin (65.7%) and gentamicin (63%). According to Kaushik et al, the Gram-negative bacteria showed maximum sensitivity to nitrofurantoin (95.5%), amikacin (75.5%) and gentamicin (65.5%) [2].The least sensitive antibiotic among the Gram-negative bacteria in our study was ampicillin/ sulbactum (21.1%) followed by cotrimoxazole (49.5%). In a study done by Sundaramurthy et al, after beta lactum antibiotics, fluoroquinolones were the least effective drugs followed by cotrimoxazole [20].

We observed 17.5% of the *Escherichia coli* and 21% of *Klebsiella pneumoniae* to be ESBL producers. In our study, both cefotaxime-clavulanic acid and ceftazidime-clavulanic acid identified ESBL producers equally, whereas in other studies cefotaxime-clavulanic acid identified more number of ESBL producers compared to ceftazidime-clavulanic acid [19,21,22]. ESBL strains apart from being resistant to third generation cephalosporins also showed more than 50% resistance to ampicillin/sulbactum, amikacin, gentamcin, ofloxacin and cotrimoxazole.

The important risk factors associated with ESBL producing organisms are prolonged hospital stay, long term usage of antibiotics, severe illness and catheterisation [23]. ESBL producing organisms do not respond to the empirical therapy of cephalosporins which leads to increased risk of morbidity and mortality and also increase in the cost of treatment [20]. Carbapenams, nitrofurantoin, piperacillin-tazobactum showed potent antibacterial activity against ESBL producing isolates which was similar to the results of other studies [24, 25].

Staphylococcus aureus 18(7.3%) was the most common Gram-positive bacteria followed by Enterococcus 7 (2.7%), and *Staphylococcus saphrophyticus* 4 (1.6%). 2 strains were of MRSA. Gram positive bacteria causing UTI is usually less compared to the Gram-negative bacteria but has gained significance due to the emergence of MRSA [26].

Some studies have isolated Enterococcus species as the commonest Gram-positive bacteria causing UTI followed by CONS [27, 28]. The prevalence of different bacteria and their antibiotic resistance vary not only from place to place but also from institute to institute and this can be due to different health care settings, different antibiotic protocols and study population.

Conclusion

Knowledge of the uropathogens and their antimicrobial susceptibility pattern in an area is essential for providing effective therapy and control of UTI. Empirical therapy should be validated by culture report to prevent morbidity associated with the disease. To limit the spread of ESBL producing isolates, ESBL detection should be included in the routine antibiotic sensitivity testing. Continued surveillance, appropriate use of antibiotics and implementation of strict infection control measures are recommended to decrease ESBL production.

Importance of this study: The most common organisms causing UTI change from place to place and also their antibiotic sensitivity pattern. Hence, it was important to conduct this study in our hospital. We found that *Escherichia coli* was the most common organism causing UTI followed by *Klebsiella pneumoniae*. The Gram negative isolates were most sensitive to meropenem followed by piperacillintazobactam and nitrofurantoin, and all the Gram positive bacteria were sensitive to vancomycin, linezolid followed by nitrofurantoin. The antibiotic sensitivity pattern of the isolates will help in guiding therapy in our hospital.

Author contributions: First author has contributed in the concept and design of work, literature search, data acquisition, data analysis, manuscript preparation and manuscript review. Second author is the corresponding author and has contributed in the concept, design, literature search, data acquisition, data analysis, manuscript preparation and manuscript review. Third author has contributed in the data acquisition, manuscript editing and manuscript review.

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