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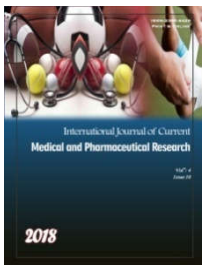

THE STUDY OF BACTERIAL PROFILE FROM URINE SAMPLES AT A TERTIARY HEALTH CARE CENTRE IN NORTH INDIA

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ABSTRACT

Objective: The purpose of the study was to determine the various bacteria etiologies inciting urinary tract infections and their antimicrobial susceptibility at a tertiary care centre in North India.
Methods: The study was performed by isolation and analysis of bacterial isolates from urine samples of patients reporting at OPD/IPD at NIMSR, Jaipur, followed by their antimicrobial susceptibility. The samples were collected and processed at the Department of Microbiology, National Institute of Medical Sciences & research from Feb. 2018-July 2018, over a period of six months. Standard Kirby-Bauer method using disc diffusion technique was employed for Antimicrobial susceptibility tests.
Results: Out of total 508 samples collected only 110 samples yielded bacterial growth while majority were found sterile. Female outnumbered males in ratio 1:1.29. The most common isolate was found to be *Escherichia coli* (41.8%) which were followed by *Klebsiella spp*. (20.9%), *CoNS* (16.4%) and *Pseudomonas spp*. (10.9%). The resistance to various antibiotics was also observed where Cefepime was found to be having highest resistance rate followed by Amoxicillin, Cefepime, Ciprofloxacin, Cotrimoxazole and Chloramphenicol. . *Escherichia coli* isolates were found to have resistance rates of82.6% and 67.3% to Amoxycillin, and Cotrimoxazole while higest resistant rate in *Klebsiella spp*. were found in Amoxicillin (78.2%) and Ciprofloxacin(65.2%).
Conclusions: *Escherichia coli, Klebsiella spp.* and *CoNS* may be the common bacterial etiological agents for UTI in the region, also the resistance rates to Cefepime, Amoxicillin and Cotrimoxazole were found high resistant while other antibiotics like Nitrofurantoin and Gentamicin were having higher sensitivity rate and thus can be considered better antimicrobials for treatment of urinary tract infection.

INTRODUCTION

Urinary tract infection (UTI) refers to a variety of infective conditions of urinary tract including asymptomatic bacteriuria to severe of the pyelonephritis with sepsis [1]. UTIs constitutes among one of the most common bacterial infections, both in the community as well as hospitals [2]. About 150 million cases are reportedly diagnosed with UTIs accounting for expenditures around 6 billion USD [1]. UTIs are the most common bacterial infections encountered by clinicians in developing countries [3]. Gram-negative bacteria are most common etiological agents of which Escherichia coli (E. coli) is the main causative agent in more than half of the cases [4,5]. Other enterobacteriaceae involved are *Klebsiella spp*., *Proteus mirabilis, Pseudomonas aeruginosa, Acinetobacter spp.*, and *Serratia spp*. and Gram-positive bacteria like *Enterococcus spp*. and *Staphylococcus spp*. are next most common agents causing UTI's [3,6].Thus causing variation in etiological agents and their susceptibility patterns in geographical regions [7-11]. Also, the dynamics of etiology and drug resistance does changes through time [12]. For the reasons above, understanding of the etiology and susceptibility patterns is needed to identify any change in susceptibility pattern of antibiotics and to recommend optimal empirical therapy for UTI [13]. Currently many studies on the prevalence and antimicrobial resistance patterns of UTIs in North India have been published [6,10,14] still, less data is available from the study area. So the purpose our study was to ascertain and compare the prevalence of bacterial uropathogens from urine samples for suspected UTI with other studies together with their antimicrobial susceptibility in the region.

MATERIALS AND METHODS

Study design*:* A total of 508 samples collected at OPD/IPD at National Instititute of Medical Sciences & research, Jaipur and were plated on blood agar, cystine lactose electrolyte-deficient medium, MacConkey agar using calibrated wire loops and were then incubated aerobically for 24 hrs at 37 °C. Culture was considered significant if yield $\geq 10^5$ CFU/ml [14]. The isolates were then subjected to antibiotic sensitivity testing on Muller Hinton Agar by standard Kirby Bauer disk diffusion method. The various antimicrobial agents tested were:

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Amikacin (30 µg), Gentamicin (10 µg), Chloramphenicol (30 µg), Cotrimoxazole (25 µg), Erythromycin (15 µg), Ciprofloxacin (5 µg), Nitrofurantoin (300 µg), Cefepime(30 µg), Amoxicillin (10 µg), Doxycycline (30 µg), Ceftriaxone (30 µg). Sensitivity and Resistance were interpreted according to Clinical laboratory Standards Institute. *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 (*S. aureus*) were used as reference strains [16].

Data analysis-- Chi-square test was employed to compare the proportion of bacterial isolates between sex and age and comparison of antimicrobial resistances. P-value of less than 0.05 was considered to indicate statistically significant difference.

RESULT

Out of total 508 samples collected only 110 samples yielded bacterial growth while majority of them were sterile. Females outnumbered the males in ratio of 1:1.29 (Table-1,Fig.-1). The most common isolate was *Escherichia coli* (41.8%) which were followed by *Klebsiella spp*. (20.9%), *CoNS* (16.4%) and *Pseudomonas spp*. (10.9%). (Table-3, Fig.-3)

Bacterial isolates were then subjected to antibiotic sensitivity testing using various antibiotics out of which Nitrofurantoin had the highest sensitivity 87(88.8%). It was closely followed by Amikacin 85(78.7%), Gentamicin 83(75.5%), Doxycycline 60 (61.2%), Erythromycin 15 (57.7%), Ceftriaxone 6(50%) and others. Antibiotics susceptibility testing showed Cefepime with highest resistance rate followed by Amoxicillin, Cefepime, Ciprofloxacin, Cotrimoxazole and Chloramphenicol (Table-4,Fig-4). *Escherichia coli* isolates were found to have resistance rates of82.6% and 67.3% to Amoxycillin, and Cotrimoxazole while highest resistant rate in *Klebsiella spp*. were found in Amoxicillin (78.2%) and Ciprofloxacin (65.2%).(Table- 5)

Among the Gram positive pathogens, *Coagulase negative Staphylococcus spp. (CoNS)* was most common isolate which was found in 16.4% of isolates with highest resistance rates in Cefepime (50%) and Cotrimoxazole(50%).(Table -5)

Table 1 Distribution of organism according to sex

Sex	No. of isolates	%
Male	48	43.64
Female	62	56.36
Total	110	100

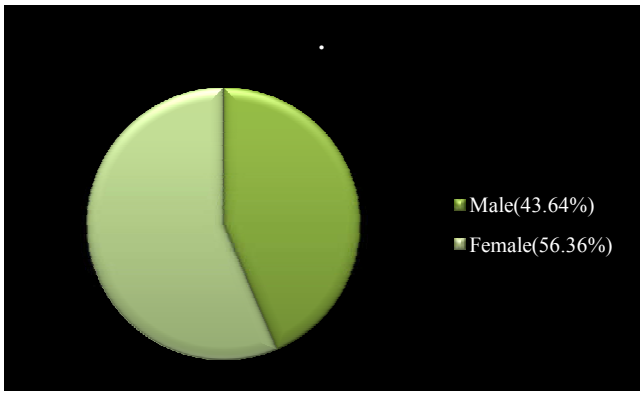

Fig 1 Distribution of organism according to sex

Table 2 Distribution of organism according to age

Age Group	No. of isolates in Male	%	No. of isolates in Female	%
0-9	5	10.4	3	4.84
10-19	3	6.25	7	11.3
20-29	16	33.3	26	41.9
30-39	4	8.33	8	12.9
40-49	6	12.5	8	12.9
50-59	6	12.5	4	6.45
60-69	4	8.33	2	3.23
>70	4	8.33	4	6.45
Total	48	100	62	100

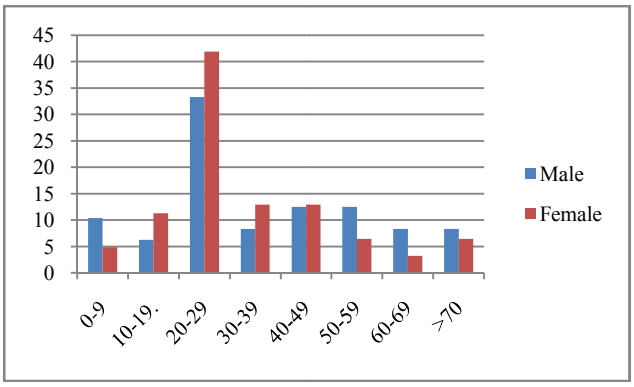

Fig 2 Distribution of organism according to age

Table 3 Distribution of bacterial isolates

Bacterial isolates	No. of Isolates	%
E. coli	46	41.8
Klebsiella spp.	23	20.9
Pseudomonas spp.	12	10.9
CoNS	18	16.4
S. aureus	6	5.45
Citrobacter spp.	1	0.91
Proteus spp.	2	1.82
Enterococcus	2	1.82
Total	110	100

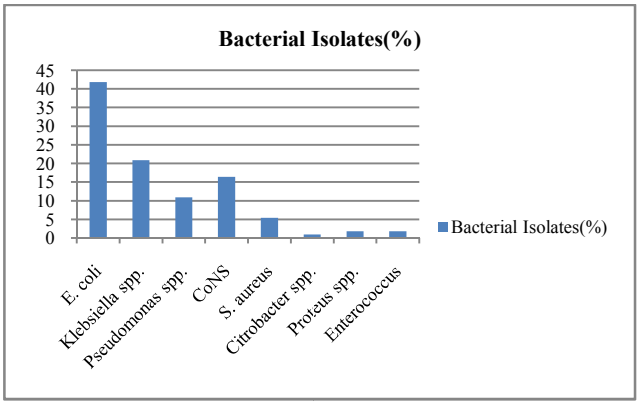

Fig 3 Distribution of bacterial isolates

Table 4 Overall susceptibility of bacterial profile

Antimicrobial	No. of microbial tested	Resistant (%)	Sensitive (%)
Amikacin	108	23(21.3)	85(78.7)
Gentamicin	110	27(24.55)	83(75.45)
Chloramphenicol	98	53(54.09)	45(45.91)
Cotrimoxazole	96	58(60.42)	38(39.58)
Erythromycin	26	11(42.30)	15(57.70)
Ciprofloxacin	110	61(55.45)	49(44.55)
Nitrofurantion	98	11(11.22)	87(88.78)
Cefepime	108	69(63.89)	39(36.11)
Amoxycillin	98	65(66.32)	33(33.68)
Doxycycline	98	38(38.78)	60(61.22)
Ceftrioxene	12	6 (50)	6(50)
Lenizolid	26	0	26(100)
Vancomycin	26	0	26(100)

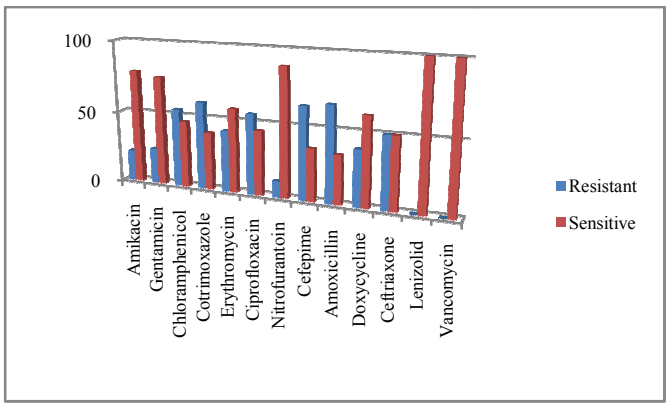

Fig 4 Overall susceptibility of bacterial profile

Nitrofurantoin as main antibiotic for empirical management of UTIs in the region.

Among the common gram negative bacterial isolates *E. coli*, and *Klebsiella spp*., showed higher resistance rates against Amoxicillin (82.6% & 78.2% respectively), while *Pseudomonas spp.* were most frequently resistant to Cefepime (91.6%). *E. coli* and *Klebsiella spp.* had highest sensitivity against Nitrofurantoin while most sensitive antimicrobial against *Pseudomonas spp.* was Amikacin. The antibiotic resistance profile was in support of previous study by Vijay Laxmi Malhotra *et, al*.(17) while was higher than the studies reported from Iran (21) and Ethiopia (4,5,22)

Table 5 Antimicrobial susceptibility of bacterial isolates

Antimicrobial agent	E. coli		Klebsiella spp.		Pseudomonas spp.		CoNS		S. aureus		Citrobacter spp.		Proteus spp.		Entero coccus	
	R	%	R	%	R	%	R	%	R	%	R	%	R	%	R	%
Amikacin	11	23.9	4	17.3	3	25.0	3	16.6	1	16.6	0	0	1	50	-	-
Gentamicin	9	19.5	5	21.7	4	33.3	5	27.7	2	33.3	0	0	1	50	1	50
Chloramphenicol	28	60.8	13	56.5	-	-	4	22.2	4	66.6	1	100	1	50	2	100
Cotrimoxazole	31	67.3	10	43.4	-	-	9	50.0	5	83.3	1	100	2	100	-	-
Erythromycin	-	-	-	-	-	-	5	27.7	4	66.6	-	-	-	-	2	100
Ciprofloxacin	30	65.2	15	65.2	6	50.0	4	22.2	4	66.6	0	0	1	50	1	50
Nitrofurantion	6	13.0	3	13.0	-	-	1	5.5	1	16.6	0	0	0	0	0	0
Cefepime	30	65.2	13	56.5	11	91.6	9	50.0	4	66.6	1	100	2	100	-	-
Amoxycillin	38	82.6	18	78.2	-	-	2	11.1	3	50.0	1	100	2	100	1	50
Doxycycline	18	39.1	11	47.8	-	-	4	22.2	2	33.3	1	100	2	100	0	0
Ceftrioxene	-	-	-	-	6	50.0	-	-	-	-	-	-	-	-	-	-
Lenizolid							0	0	0	0					0	0
Vancomycin							0	0	0	0					0	0

DISCUSSION

Although introduction of newer antimicrobials has refined the management of UTIs but the emergence of antimicrobials drug resistance among bacterial uropathogens has over shadowed the chemotherapeutic response. In our study overall isolation rate was 21.6% which was higher than the other studies reported from north India (14,17) but was less than the study by Arti Ninama et.al.(23)where isolation rate was 30%. *E. coli* was single most common isolate (41.8%) followed by *Klebsiella spp*. (21.9%), *CoNS* (16.4%), *Pseudomonas spp*. (10.9%) and *Staphylococcus aureus* (5.5%) which was in support of other previous studies (14,17). *CoNS* (16.4%) followed by Staphylococcus aureus (5.5%) were most frequently isolated Gram positive pathogens which were again in accordance to other studies but the predominance of *CoNS* was less as compared to our study (4.2%). (17)

The differences between etiology and isolation rates may be due to variations in geographical regions (5,18) and methods of identifications(13). The number of samples and isolation rates were significantly higher in female patients (56.4%) than males (43.6%). Similar results were also reported in other studies across the world (6,9,14,17) which may be because of differences in urinary tract physiology and anatomy in females where the length of urethra is less (19). Other reasons which may contribute to higher rates of UTI in females may be sexual trauma, pregnancy and child birth. Also the majority of the isolates were from younger (20-29 yrs) age group, which was in accordance with the other studies from the region (20).
The overall bacterial susceptibility profile was highest for Nitrofurantoin (88.8)% followed by Amikacin (78.7%) which was in support of another study done by Mulugeta Kibret *et al*.(24). This finding may be helpful in recommending

Among the Gram positive isolates *CoNS* and *Staphylococcus aureus* showed highest antimicrobial susceptibility against Vancomycin (100%) and Lenizolid (100%). The resistant rates for *CoNS* was highest against Cotrimoxazole (50%) and Cefepime (50%), On the otherhand resistance rates for *Staphylococcus aureus* was highest against Cotrimoxazole (83.3%%) and Chloramphenicol (66.6%). Another study by Jain S *et. al*.(14), also showed 100% sensitivity of vancomycin and lenizolid against gram positive uropathogens like *CoNS* and *Staphylococcus aureus*(14). Thus the present study support Nitrofurantoin, Amikacin, Gentamicin as antimicrobials of choice for gram negative uropathogens while for gram positive agents, the study recommends Vancomycin, Linezolid, Amikacin and Gentamicin for first line therapy. However frequency of bacterial uropathogens and their antimicrobial susceptibility profile varies with time and geographical regions, so periodic monitoring of etiological agents and their susceptibility profile is required for appropriate diagnosis and management of urinary tract infections.

CONCLUSION

Urinary tract infection is among the most frequently encountered infective conditions by physicians but the dynamicity in etiological profile, antimicrobial susceptibility and emergence of resistance is stumbling the management of the disease. The emergence of resistance to commonly prescribed antibiotics like Fluoroquinolone, Aminoglycoside and Beta-lactam antibiotics is limiting the options available for empirical therapy.

As a result there is significant increase in patient morbidity, duration of infection, along with the treatment costs. However with prior urine culture and sensitivity testing and judicious use of antimicrobials, therapeutic management and emergence

of resistance can be restrained. Also regular monitoring of uropathogens and their sensitivity profile is imperative for faming of antibiotic prescribing guidelines for any geographical region.

Conflicts of interest: None

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