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Isolation and identification of pathogenic bacteria causing urinary tract infection and their antibiotic susceptibility pattern in a tertiary care hospital North-West Rajasthan

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Abstract

Background: The aim of present study was to record the distribution of bacterial strains isolated from UTI patients and their resistance pattern against commonly used antibiotics at our setting was studied.

Methods: Hospital based cross-sectional study was conducted on 250 patients in age group 1 to 65 years admitted in hospital and OPD patients with a probable diagnosis of urinary tract infection. Patients with symptoms like fever, abdominal pain, dysuria, smelly urine were subjected for urine routine and microscopic examination. Verbal consent was taken from the parents or guardians before enrolling them in the study.

Results: Majority of cases were from middle socio-economic status, female belong to urban area. Fever (64.2%) was most common presentation followed by abdominal symptoms (32%) and urinary symptoms (23.2%). Uncomplicated UTI was found in 206(82.4%) cases and 26(10.4%) cases had complicated UTI. 26 cases blood culture showed growth of organism. Urine culture was sterile in 116(46.4%) cases, Gram-negative bacteria were grown in 41.6% cases, Gram-positive in 4.4%, fungal infection in 5.2% and mixed organisms grown in 2.4% cases. E. coli was grown in 70(52.2%) cases, Klebsiella spp. in 16(11.9%), Enterococcus sapp. in 13(9.7%) cases, Candida spp. was grown in 13(9.7%) cases, Acinetobacter spp. and MRSA in 4(3.0%) cases each, CONS and MSSA in 3(2.4%) cases each. Klebsiella and E.Coli were more sensitive(90%) to Piperacillin+ Tazobactam and cefepime followed by meropenem/imipenem(85%). Enterococcus and other Gram positive organisms were more more sensitive (95.8%) to Amoxyclav and Vancomycin followed by linezolid(91.6%). Pseudomonas strains was more sensitive to Piperacillin+ Tazobactam (90.91%) followed by Cefoperazone(72%)

Conclusion: This study concludes that pathogens causing UTI have developed resistance, even to newer antibiotics, probably due to overuse of antibiotics. To

overcome this problem, irrational antibiotic therapy should be limited.

Keywords: UTI, Antibiotic, Sensitivity, Resistance

Introduction

Urinary Tract Infection is classified as the most common occurring nosocomial bacterial infection in human populations around the world. UTI is a condition caused by pathogenic invasion of the epithelium, which lines the urinary tract from the minor calyx to prostatic urethra. The proliferation of bacteria in the urothelium can be asymptomatic or symptomatic, which causes inflammatory response and symptomatic case characterized by a wide range of symptoms including, fever, lethargy, anorexia and vomiting. However, both genders are susceptible to this type of infection, but women are more, as their reproductive anatomy and physiology are more sensitive. Half of all women by 32 years age had experienced at least an infection history. The developing renal cortex in young children is vulnerable to renal scarring resulting in hypertension and chronic renal failure. These morbidities in adults often have their origin in childhood. A clinically suspected case of UTI should be defined and documented with urine culture report. After the diagnosis of UTI, its category should be defined. This helps in guiding a clinician about the appropriate radio/nuclear imaging evaluation, choice of antimicrobial agent, duration of treatment and need of chemoprophylaxis. Even a single confirmed UTI should be taken seriously.[1-3]

The most common pathogens causing UTI are Escherichia coli, Klebsiella species, Enterococcus species., Streptococcus agalactiae, Proteus mirabilis, Staphylococcus saprophyticus, Viridans streptococci, Pseudomonas aeruginosa, CONS species to list a few. Among these microbes, E. coli contributes to about 86% of the infection of UT. UTI can occur in all seasons. However, a spike in the infection can be observed during warm summer. Uropathogenic E. coli (UPEC) produce exotoxins such as hemolysin, cytotoxic factor type 1 (CNF1), and colonization factors that have made it a major pathogen causing UTI [4,5]

Materials And Methods

Study design: Hospital based cross-sectional study.

Study place: Department of microbiology, S.P. Medical College and P.B.M associated group of Hospital, Bikaner

Sample size: Sample size of this study was 250 cases when prevalence of UTI was 5% and epi info software was used. 5% prevalence of UTI according to Patel et al[25].

Sampling Method: Simple random sampling. 250 samples were selected randomly.

Inclusion criteria: All patients in age group 1 to 65 years admitted in hospital and OPD patients with a probable diagnosis of urinary tract infection. Patients with symptoms like fever, abdominal pain, dysuria, smelly urine were subjected for urine routine and microscopic examination. Verbal consent was taken from the parents or guardians before enrolling them in the study. Patients willing to participate in the study.

Exclusion criteria: Patients less then 1 year or greater then 65 years and Patients not willing to participate in our study was excluded. Patients on antibiotics were also excluded from our study.

Data Collection: Patients presenting with urinary symptoms (dysuria, urgency, frequency, incontinence, hematuria and suprapubic pain) and those with fever without focus was enrolled in the study. History was noted and patients was clinically examined. Complicated UTI (involvement of upper urinary tract) was diagnosed if there was presence of any one or all of the following- fever >39^{0}C, systemic toxicity, persistent vomiting, dehydration, renal angle tenderness and raised serum

creatinine. Recurrent UTI was considered if there was previous history of one or more episodes of proven UTI.

Wet mount microscopy of urine was done to detect pyuria, hematuria and presence of any bacteria. Clean catch midstream urine samples was collected. Urine culture was done on Mac Conkey agar, Nutrient agar and Hichrome UTI agar , blood agar with a calibrated loop. A growth of greater than 10^5 colony forming units/ml of a single organism for midstream urine samples and greater than $5x10^4$ colony forming units/ml for samples obtained by catheterization was considered significant bacteriuria and UTI. The antibiotic sensitivity test was done on Mueller-Hinton agar by Kirby-Bauer disc diffusion test and ESBL (Extended Spectrum Beta Lactamase) production was detected by double disc synergy tests as per the recent Clinical and Laboratory Standard Institute (CLSI) guidelines [46].

Data Analysis

To collect required information from eligible patients a pre-structured pre-tested Proforma was used. For data analysis statistical software SPSS was used and data was analyzed with the help of frequencies, figures, proportions, measures of central tendency, appropriate statistical test.

Observations

This Hospital based cross-sectional study was carried out at Department of microbiology, S.P.Medical College and P.B.M associated group of Hospital, Bikaner.A total of 250 samples were selected randomly from Dec. 2021 to Nov.2022.

Table 1: Distribution of cases according to urine culture

Organism grown	No. of Cases	%
Gram Negative		
E. Coli	70	28.0
Klebsiella species	16	6.4
Enterococcus species	13	5.2
Acinetobacter species	4	1.6
Proteus species	1	0.4
Gram Positive		
MRSA	4	1.6
MSSA	3	1.2
CONS	3	1.2
Staphylococcus aures	1	0.4
Fungal Infection		
Candida albicans	8	3.2
Candida non-albicans	5	2.0
Mixed		
E. Coli & MRSA	1	0.4
E. Coli & Candida Non-albicans	1	0.4
E. Coli & Acinetobacter species	1	0.4
E. Coli & Klebsiella species	1	0.4
E. Coli & Enterococcus	1	0.4
Klebsiella species & Candida albicans	1	0.4
Sterile	116	46.4
Total	250	100

Urine culture was sterile in 116(46.4%) cases, Gram negative bacteria grown in 41.6% cases, Gram positive in 4.4%, fungal infection in 5.2% and mixed infection grown in 2.4% cases. 70(28%) cases had E. coli, Klebsiella was present in 16(6.4%) cases, enterococcus was present in 14(5.6%), Candida albicans was present in 8(3.2%) cases, Candida non albicans was present 5(2.0%)cases, MRSA and Acinetobacter was present in 4(1.6%) cases each, MSSA and CONS was present in 3(1.2%) cases each, more than one species grown in 6(2.4%) cases while, Proteus and Staphylococcus was present in 1 case each.

Table 2: Mono microbial vs polymicrobial samples

No of organism	No of culture positive cases	Percentage
Single organism	245	98.00%
Multi organism	5	2.00%

Out of 250 cases in 5 cases multiorganism growth was seen and in 245 cases single organism growth was seen.

Table 3: Antifungal sensitivity of fungal isolates grown in urine culture

Fungal	Fluconazole		Nystatin		Ketoconazole		Itraconazole		Amphotericin	
	S	R	S	R	S	R	S	R	S	R
Candida albicans (8/13)	5	3	4	4	2	6	5	3	1	7
Candida non albicans (5/13)	2	3	3	2	2	3	4	1	3	2

In our study out of 8 cases of candida albicans, 5 cases were sensitive to fluconazole , 4 cases to nystatin, 2 cases to ketoconazole, 5 cases to itraconzole, 1 case to amphotericin. Out of 5 cases of candida non albicans, 2 cases were sensitive to fluconaole , 3 cases to nystatin, 2 cases to ketoconazole, 4 cases to itraconzole and 3 cases to amphotericin.

Table 4: Antibiotic susceptibility pattern of commonly grown Gram-negative organisms(E.Coli & Klebsiella spp.)

Antibiotic	Sensitivity	E. Coli (n=70)		Klebsiella (n=16)	
		No.	%	No.	%
Nitrofurantoin	Sensitive	42	60.00	40	57.14
	Resistance	28	40.00	30	42.76
Meropenem/ Imipenem	Sensitive	60	85.71	58	82.85
	Resistance	10	14.29	12	17.15
Cefotaxime	Sensitive	58	82.85	58	82.85
	Resistance	12	17.15	12	17.15
Cotrimoxazole	Sensitive	45	64.28	49	70.00
	Resistance	25	35.72	21	30.00
Ampicillin	Sensitive	40	57.14	38	54.28
	Resistance	30	42.86	32	45.72
Cefoperazone	Sensitive	63	90.00	60	85.71
	Resistance	7	10.00	10	14.29
Amikacin/gentamycin	Sensitive	45	64.28	45	64.28
	Resistance	25	35.72	25	35.72
Piperacillin+ Tazobactam	Sensitive	63	90.00	63	90.00

	Resistance	7	10.00	7	10.00
Ceftriaxone	Sensitive	42	60.00	45	64.28
	Resistance	28	40.00	25	35.72
Ofloxacin	Sensitive	35	50.00	35	50.00
	Resistance	35	50.00	35	50.00
Norfloxacin	Sensitive	49	70.00	49	70.00
	Resistance	21	30.00	21	30.00
Doxycycline	Sensitive	28	40.00	28	40.00
	Resistance	42	60.00	42	60.00
Cefepime	Sensitive	63	90.00	63	90.00
	Resistance	7	10.00	7	10.00

In our study Klebsiella and E.Coli were more sensitive(90%) to Piperacillin+ Tazobactam and cefepime followed by meropenem/imipenem(85%)

Table 5: Antibiotic susceptibility pattern of Enterococcus spp. and Other Gram-positive organisms

Antibiotic	Sensitivity	Enterococcus and other gram positive organism (n=24)	
		No.	%
Ampicillin	Sensitive	20	83.33
	Resistance	4	16.67
Amoxy clav	Sensitive	23	95.83
	Resistance	1	4.17
Vancomycin	Sensitive	23	95.83
	Resistance	1	4.17
Cefotaxime	Sensitive	22	91.67
	Resistance	2	15.38
Cotrimoxazole	Sensitive	9	37.50
	Resistance	15	62.50
Linezolid	Sensitive	22	91.67
	Resistance	2	15.38
Cefoperazone	Sensitive	6	25.00
	Resistance	18	75.00
Amikacin	Sensitive	10	41.67
	Resistance	12	58.33
Ciprofloxacin	Sensitive	16	66.67
	Resistance	8	33.33
Azithromycin	Sensitive	20	83.33

	Resistance	4	16.67
Piperacillin+ Tazobactam	Sensitive	20	83.33
	Resistance	4	16.67
Ceftriaxone	Sensitive	6	25.00
	Resistance	18	75.00
Doxycycline	Sensitive	6	25.00
	Resistance	18	75.00
Cefepime	Sensitive	6	25.00
	Resistance	18	75.00
Clindamycin	Sensitive	2	8.33
	Resistance	22	91.67

In our study Enterococcus and other Gram positive organisms were more more sensitive (95.8%) to Amoxyclav and Vancomycin followed by linezolid(91.6%)

Table 6. Antibiotic susceptibility pattern of Pseudomonas organisms

Antibiotic	Sensitivity	Pseudomans (n=11)	
		No.	%
Piperacillin+ Tazobactam	Sensitive	10	90.91
	Resistance	1	9.09
Meropenem/ Imipenem	Sensitive	7	63.63
	Resistance	4	27.27
Aztreonam	Sensitive	8	72.73
	Resistance	3	27.27
Cefotaxime	Sensitive	7	63.63
	Resistance	4	36.37
Cotrimoxazole	Sensitive	1	9.09
	Resistance	10	90.91
Amikacin	Sensitive	2	18.18
	Resistance	9	81.82
Cefoperazone	Sensitive	8	72.73
	Resistance	3	27.27
Toberamycin	Sensitive	6	54.54
	Resistance	5	45.46
Ciprofloxacin	Sensitive	6	54.54
	Resistance	5	45.46

Ceftriaxone	Sensitive	7	63.63
	Resistance	4	36.37
Ofloxacin	Sensitive	5	45.45
	Resistance	6	54.55
Norfloxacin	Sensitive	6	54.55
	Resistance	5	45.45
Doxycycline	Sensitive	2	18.18
	Resistance	9	81.82

In our study pseudomonas strains was more sensitive to Piperacillin+ Tazobactam (90.91%) followed by Cefoperazone (72%)

Discussion

Urine culture was sterile in 116(43.2%) cases, Gram-negative bacteria were grown in 41.6% cases, Gram-positive in 4.4%, fungal infection in 5.2% and mixed infection seen in 2.4% cases. 70(28%) cases had E. coli, Klebsiella spp. was grown in 16(6.4%) cases, Enterococcus spp. was grown in 14(5.6%), Candida albicans was grown in 8(3.2%) cases, Candida non albicans was grown in 5(2.0%)cases, MRSA and Acinetobacter spp. were grown in 4(1.6%) cases each, MSSA and CONS were grown in 3(1.2%) cases each. Proteus spp. and Staphylococcus aureus was grown in 1 case each. Out of total 134 culture positive isolates monomicrobial growth was seen in 98% samples while polymicribial growth was seen in 2.4% samples

According to our study urine culture was positive in 53.6% cases. In studies by other authors, Singh et al[6] (45.2%) cases, Malla et al[7] (57.0%), Mashouf et al[8] (34.2%), Rehman et al[9] (37.5%) had positive urine culture, which is in line with our study. While unlike to our study Karishnan et al[10] (16.4%), Patel et al[11] (27.1%), Taneja et al[12] (28.3%) shows low detection rate.

Mashouf et al[8] observed that the most common isolates were E. coli (57.4%), K. pneumoniae (9.7%), E. aerogenes (7.0%), S. aureus (5.8%), C.freundii (5.1%), P. mirabilis (4.5%), P. aeruginosa (3.2%), A. baumannii (2.2%), coagulase-negative Staphylococcus (3.2%) and E. faecalis (1.9%), which is similar with our study. In study by Taneja et al[12] common uropathogens isolated were Escherichia coli (47.1%), Klebsiella spp. (15.6%), Enterococcus fecalis (8.7%). Singh et al[6] observed that E. coli (78.7%) was the most common organism found followed by Klebsiella pneumonie (13.1%),

Out of total 134 culture positive cases, E. coli was present in 70(52.2%) cases, Klebsiella spp. in 16(11.9%), Enterococcus spp. in 13(9.7%) cases, Candida infection was present in 13(9.7%) cases, Acinetobacter and MRSA in 4(3.0%) cases each, CONS and MSSA in 3(2.4%) cases each. Out of 69 culture positive females, in 38 cases (55.1%) E. coli was grown, Klebsiella spp. in 10(14.5%) cases and Enterococcus spp. in 5(7.2%) cases were grown. Out of 65 culture positive males E. coli was grown in 32(49.2%) cases, Klebsiella spp. in 6(9.2%) cases and Enterococcus spp. in 8(13.3%) cases were grown . Proteus spp. and Staphylococcus aureus were grown in 1 case each while more than 1 organisms grown in 6(4.2%) cases.

Farajnia et al[13] observed that E. coli is the predominant isolated pathogen from both sexes, it occurred significantly more frequently in female (76.5% in women compared to 62.4% in male), whereas the prevalence of UTI due to Klebsiella pneumoniae and Pseudomonas aeruginosa were higher in male than in female (22.6%

and 7.5% in male compared to 9.9% and 1.4% in female, respectively). In other study by A Sharma et al[14] E.coli was present in 67.5% cases. Fan et al[15] observed that gram negative bacilli were predominant pathogenic bacteria, accounting for 79.0% of the cases, and Escherichia coli was most commonly found (56.2%).

In our study prevalence of UTI due to Enterococcus spp. was higher in male (12.3%) than female (7.2%). Klebsiella spp. was isolated in 11.9% cases in ours study. Similar finding was also noted in various studies where Klebsiella was isolated in 15.7% cases by Taneja et al[12]

Conclusion

UTI is a common illness. This study shows age and gender distribution in accordance to available literature. Females were more commonly affected than males. Fever being most common presenting symptom followed by vomiting and pain abdomen. There were 53.6% cases of significant bacteriuria and 11.2% cases of associated bacteraemia. E. coli was the most common organism cultured in the urine, it represented 52.2% of urine isolates. The second most prevalent bacteria was Klebsiella species. (11.9%). This study concludes that pathogens causing UTI have developed resistance, even to newer antibiotics, probably due to overuse of antibiotics. To overcome this problem, irrational antibiotic therapy should be limited. Large scale studies are required to monitor the pattern of antibiotic sensitivity and resistance to formulate appropriate empiric pharmacotherapy for UTI. Continuous monitoring of changes in bacterial pathogens causing UTI and antibiotic sensitivity in each area should be done to improve the knowledge of physicians for effective treatment of urinary tract infections. The best choice of antibiotics in our study for empirical treatment of UTI before urine culture reports are meropenem, ciprofloxacin, cefotaxime, amikacin for sick patients requiring parenteral therapy. For outpatients nitrofurantoin and cotrimoxazole can be given as drug of first choice. In majorities of cases uropathogens were resistant to ampicillin, amoxiclav, quinolones and nalidixic acid.

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