



A STUDY OF ANTIBIOTIC RESISTANCE PATTERN IN UROPATHOGENS AT TERTIARY CARE HOSPITAL , RAJASTHAN

Microbiology

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ABSTRACT

Objectives: The objective of this study is to determine the distribution and antibiotic susceptibility patterns of bacterial strains isolated from patients with urinary tract infections (UTI) at tertiary care hospital in, Rajasthan, as well as identification Multi-drug resistance (MDR) organisms in the population of different uropathogens.

Materials and Methods: Samples received by the bacteriology section of microbiology department, from various wards of Hospital will be processed. Standard diagnostic procedures will be followed, using media and stains approved in the laboratory for aerobic culture. Relevant baseline information of the patient will be noted. Internal quality control methods will be observed to ensure correctness of the results. The data thus created will be documented and analysed.

Results: The overall prevalence of UTI in both male and female patients was found to be 65.28%. Among 210 samples, 137 urine samples showed the significant bacterial growth which were comprised of 42 (30.66%) samples from males and 95 (69.34%) from females. Out of the 134 culture isolates, *Escherichia coli* was the most common 55 (26.19%) followed by *Klebsiella pneumoniae* (10.95%), *Pseudomonas aeruginosa* (6.67%), *Enterococcus sp.* (5.34%), *Staphylococcus aureus* (4.76%). The sensitivity was observed as 46.19% to Amikacin, 42.86% to Norfloxacin, 42.38% to Nitrofurantoin, 40.48% to Gentamicin, 30.95% to Meropenem, 25.71% to Ciprofloxacin, 20.95% to Piperacillin-tazobactam and 19.05% to Linezolid, 18.57% to Co-trimoxazole, 17.14% to Amoxicillin-clavulanic acid, 10.4% to and 16.19% to Chloramphenicol and Cefotaxime. On the other hand, as far as the resistance of uropathogens towards antibiotics is concerned, it is found as – 29.05% to Ciprofloxacin, 22.38% to Ampicillin, 21.43% to Amoxicillin-clavulanic acid, 20.47% to Co-trimoxazole, 17.61% to Cefotaxime, 12.86% cepipime and 11.90% to Erythromycin, Penicilin.

Conclusion: Routine monitoring of resistance as well sensitivity patterns is necessary, which will help the clinicians in the empirical treatment of UTI and also for preparation of an antibiotic policy. The high rate of resistance to Ciprofloxacin, Ampicillin, Amoxicillin-clavulanic acid, Co-trimoxazole, Cefuroxime, Cefazolin, Cefotaxime, of urinary isolates, precludes the use of these commonly used antibiotics for empiric treatment of UTI in India, where as sensitivity towards amikacin, norfloxacin, nitrofurantoin recommends there use for the treatment.

KEYWORDS

Urinary tract infection, resistance, sensitivity, Routine monitoring.

INTRODUCTION

A urinary tract infection (UTI) is an infection from microbes. These are organisms that are too small to be seen without a microscope. Most UTIs are caused by bacteria, but some are caused by fungi and in rare cases by viruses. UTIs are among the most common infections in humans. It increased morbidity in both hospitalized as well as outpatients. Although, a variety of organisms are responsible for UTI, *Escherichia coli* and other members of the family Enterobacteriaceae account for a large majority of UTIs¹

A UTI can happen anywhere in the urinary tract. Your urinary tract is made up of your kidneys, ureters, bladder, and urethra. Most UTIs only involve the urethra and bladder, in the lower tract. However, UTIs can involve the ureters and kidneys, in the upper tract. Although upper tract UTIs are rarer than lower tract UTIs, they're also usually more severe.

Risk factors include female anatomy, sexual intercourse, diabetes, obesity, and family history². Although sexual intercourse is a risk factor, UTIs are not classified as sexually transmitted infections (STIs)¹. Kidney infection, if it occurs, usually follows a bladder infection but may also result from a blood-borne infection⁵. Diagnosis in young healthy women can be based on symptoms alone. In those with vague symptoms, diagnosis can be difficult because bacteria may be present without there being an infection⁶. In complicated cases or if treatment fails, a urine culture may be useful.

Increasing drug resistance in bacterial uropathogens is an important and emerging public health problem. The distribution of antimicrobial susceptibility data of UTI-causing microorganisms changes from place to place. Generally, the antimicrobial treatment is initiated before the culture sensitivity results are available which may lead to the frequent misuse of antibiotics. The current knowledge of the organisms causing UTI and their antimicrobial susceptibility pattern is a necessity, in order to ensure appropriate empirical therapy. In view of the increasing antimicrobial resistance among bacterial isolates

causing UTI, this study was undertaken at a tertiary care centre to determine the spectrum of microorganisms responsible for UTI and their antimicrobial resistance pattern.

About 150 million people developed a urinary tract infection each year². They are more common in women than men³. In women, they are the most common form of bacterial infection. Up to 10% of women have a urinary tract infection in a given year and half of women having at least one infection at some point in their lives³. They occur most frequently between the ages of 16 and 35 years. Recurrences are common³. Urinary tract infections have been described since ancient times with the first documented description in the Ebers Papyrus dated to c. 1550 BC

They are the most common cause of hospital acquired infections accounting for approximately 40%. Rates of asymptomatic bacteriuria with age from 2% to 7% in women of child bearing age to as high as 50% in elderly women in care homes. Rates of asymptomatic bacteria in the urine among men over 75 are between 7-10%.

AIMS AND OBJECTIVE

Aim: To find various bacterial pathogens present in urine and to determine their antibiotic sensitivity and resistance pattern against the commonly used standard antibiotics.

Objective:

In View of the emerging drug resistance amongst bacteria, therapy should only be advocated, as far as possible, after culture and sensitivity has been performed. This would not only help in the proper treatment of the patients but would also discourage the indiscriminate use of the antibiotics and prevent further development of bacterial drug resistance

- To isolate uropathogens causing UTI
- To study sensitivity and resistance pattern of isolated pathogens for the antibiotics,

MATERIAL AND METHOD

Sample collection: Sample will be collected in laboratory in sterile containers, freshly voided mid –stream urine from symptomatic patients. Container bearing the patients name, age sex etc.

Initial examination: The appearance of a specimen of urine and that of any appreciable amount of urine sample in a container, turbidity & color are observed.

Characterization of Bacterial Isolates: The urine sample will be cultured on Cysteine Lactose Electrolyte Deficient (CLED) medium. Inoculation of CLED medium is done using a calibrated loop of 0.001 ml as per standard protocol. Please will be incubated at 37 ° c for 24 hours in aerobic environment. Single positive culture isolates of CFU > 10⁵ the characterization of bacterial isolates will be based on standard microbiological methods “ Mackie and Mc Cartney's practical medical microbiology 14th edition “ Identification of isolates will be done based on colony morphology, motility ,catalase test ,oxidase test,

Coagulase test and biochemical tests like triple sugar iron agar hydrogen sulfide test carbohydrate fermentation test, phenylalanine deaminase test, Methyl red test , Nitrate reduction test ,Voges proskauer ,citrate utilization test and Indole test.

Antibiotics Susceptibility Testing

Susceptibility of isolates to different antibiotics will be tested by following Kirby Bauer disc diffusion method using Muller Hinton Agar against selected antibiotics. Inhibition zone size has will be interpreted using standard recommendation of National Committee Laboratory Standards now known as Clinical Laboratory Standard Institute (CLSI)

RESULT

The overall prevalence of UTI in both male and female patients was found to be 65.28%. Among 210 samples, 137 urine samples showed the significant bacterial growth which were comprised of 42 (30.66%) samples from males and 95 (69.34%) from females. Table 1 shows the distribution of samples. Out of total 210 samples 55(23.81%) were sterile i.e there was no bacterial growth observed in the sample. 22(10.48%) showed mixed growth i.e. growth of more than one organisms.

Out of the 134 culture isolates [Table 3], *Escherichia coli* was the most common 55 (26.19%) followed by *Klebsiella pneumoniae* (10.95%), *Pseudomonas aeruginosa* (6.67%), *Enterococcus sp.* (5.34%), *Staphylococcus aureus* (4.76%).. The antibiotic sensitivity pattern was analyzed for all the bacterial isolates together and drugs are sorted within the table in order of their sensitivity [Table 4]. On the basis of antibiotic sensitivity pattern analysis, all bacterial isolates have shown their sensitivity and resistance towards specific antibiotics. The sensitivity was observed as 46.19% to Amikacin, 42.86% to Norfloxacin ,42.38% to Nitrofurantoin ,40.48% to Gentamicin, 30.95% to Meropenem , 25.71% to Ciprofloxacin, , 20.95% to Piperacillin-tazobactam and 19.05% to Linezolid,18.57% to Co-trimoxazole, 17.14% to Amoxicillin-clavulanic acid, 10.4% to and 16.19% to Chloramphenicol and Cefotaxime. On the other hand, as far as the resistance of uropathogens towards antibiotics is concerned, it is found as – 29.05% to Ciprofloxacin, 22.38% to Ampicillin, 21.43% to Amoxicillin-clavulanic acid, 20.47% to Co-trimoxazole, 17.61% to Cefotaxime, 12.86% cefipime and 11.90% to Erythromycin, Pencillin.

It was observed that in both sexes, the maximum number of uropathogens were isolated from the patients in the age group 31-40 years, 32 females and 17 males, followed by age group 41-50 years, 21 females and 10 males. It is evident from the table 2, that UTI is frequently found in females. Although, urinary tract infections affect both sexes, it is a well-known fact that it is more common in females as compared to males because of shorter urethra in females which is less effective for preventing bacterial entry and closer proximity of urethral meatus and anal opening, sexual intercourse, inconsistency and bad toilets

From table 3 it is clearly evident that the organism, isolated from sampled units, responsible for causing the urinary tract infection is found *Escherichia coli*. It is evident from above table that the organism highly responsible for urinary tract infections is *Escherichia coli*,

meaning by that it is causing factor for UTI in 6.67% male and 19.52% females, which is 26.19% of the samples. The other bacteria which were isolated in the sample but not highly responsible for causing urinary tract infections in the youth population, they are *Klebsiella pneumoniae* (10.95%), *Pseudomonas aeruginosa* (6.67%), *Enterococcus sp.* (5.34%), *Staphylococcus aureus* (4.76%).

Klebsiella pneumoniae is responsible for causing urinary tract infection in 10.95% population which is highest after *Escherichia coli*, the next highest responsible uropathogen is found *Pseudomonas aeruginosa* (6.67%), followed by *Enterococcus sp.* (5.34%) and *Staphylococcus aureus* (4.76%).

Table 4 shows the antibiotic sensitivity pattern in the studied sample. By observing drug sensitivity pattern in sampled units, it is found that all bacterial isolates have shown their sensitivity and resistance towards specific antibiotics, as far as sensitivity is concerned it was observed as - The sensitivity was observed as 46.19% to Amikacin, 42.86% to Norfloxacin, 42.38% to Nitrofurantoin 40.48% to Gentamicin, 30.95% to Meropenem , 25.71% to Ciprofloxacin, , 20.95% to Piperacillin-tazobactam and 19.05% to Linezolid, Co-trimoxazole, 17.14% to Amoxicillin-clavulanic acid, 16.19% to Chloramphenicol and Cefotaxime, 13.81% to Vancomycin, 12.38% to Co-trimoxazole.

On the other hand, as far as the resistance of uropathogens towards antibiotics is concerned, it is found as – 29.05% to Ciprofloxacin, 22.38% to Ampicillin, 21.43% to Amoxicillin-clavulanic acid, 20.47% to Co-trimoxazole, 17.61% to Cefotaxime, 12.86% cefipime and 11.90% to Erythromycin , Pencillin. 11.43% Clindamycin, 10.95% Norfloxacin, 10% Gentamicin, 9.04% Amikacin. Resistance towards other pathogens contributes as 7.14%, 6.67%, 5.71%, 4.75%, 3.81%, 3.33%.

Table.1 Distribution of Study Group (N=210)

	Male	Female	Total
Total Samples	64 (30.47%)	146(69.52%)	210
Sterile	18(8.57%)	33(15.71%)	55(23.81%)
Mixed Growth	4(1.90%)	18(8.57%)	22(10.48%)
Samples positive for uropathogens	42(30.66%)	95(69.34%)	137(65.25%)
Total	54	146	210

Table.2 Age and sex-wise distribution of positive urine cultures

Age group (years)	Female		Male	
	Number	%	Number	%
20-30	19	20	7	16.67
31-40	32	33.68	17	40.48
41-50	21	22.10	10	23.81
51-60	12	12.63	5	11.90
>60	11	11.58	3	7.14
Total	95		42	

Table.3 Organism Isolated For Urinary Tract Infection (N=210)

S.No.	Organism Isolated	Count Value		Total
		M (%)	F (%)	
1	Sterile	18(8.57%)	33(15.71%)	51(24.28%)
2	Mixed Growth	4(1.90%)	18(8.57%)	22(10.48%)
3	<i>Escherichia Coli</i>	14(6.67%)	41(19.52%)	55(26.19%)
4	<i>Klebsiella Spp</i>	9(4.29%)	14(6.67%)	23(10.95%)
5	<i>Pseudomonas aeruginosa</i>	4(1.90%)	10(4.76%)	14(6.67%)
6	<i>Enterococcus sp.</i>	4(1.90%)	7(3.33%)	11(5.34%)
7	<i>Staphylococcus aureus</i>	3(1.43%)	7(3.33%)	10(4.76%)
8	<i>Candida Albicans</i>	1(0.48%)	4(1.90%)	5(2.38%)
9	<i>Citrobacter</i>	1(0.48%)	4(1.90%)	5(2.38%)
10	Methicilin Sensitive Cops	2(0.95%)	3(1.43%)	5(2.38%)
11	<i>Candida Non Albicans</i>	1(0.48%)	3(1.43%)	4(1.90%)

12	Proteus Mirabilis	0	1(0.48%)	1(0.48%)
13	Non Fermenter	2(0.95%)	0	2(0.95%)
14	Shigella	1(0.48%)	0	1(0.48%)
	Total	64(30.48%)	146(69.52%)	210(100%)

Table.4 Drug Sensitivity Pattern in Patients (N=210)

S. No	Drugs	Count Value			Total
		NR(%)	R(%)	S(%)	
1	Amikacin	94(44.76)	19(9.04)	97(46.19)	210
2	Norfloxacin	97(46.19)	23(10.95)	90(42.86)	210
3	Gentamicin	104(49.52)	21(10)	85(40.48)	210
4	Ampicillin	148(70.48)	47(22.38)	15(7.14)	210
5	Amoxicillin+Clavulanic Acid	129(61.43)	45(21.43)	36(17.14)	210
6	Piperacillin-Tazobactam	154(73.33)	12(5.71)	44(20.95)	210
7	Nitrofurantoin	106(50.47)	15(7.14)	89(42.38)	210
8	Cefipime	162(77.14)	27(12.86)	21(10)	210
9	Ceftazidime	193(91.90)	8(3.81)	9(4.28)	210
10	Meropenem	133(63.33)	12(5.71)	65(30.95)	210
11	Ciprofloxacin	95(46.19)	61(21.05)	54(25.71)	210
12	Co-Trimoxazole	141(67.14)	43(20.47)	26(12.38)	210
13	Vancomycin	174(82.86)	7(3.33)	29(13.81)	210
14	Linezolid	163(77.62)	7(3.33)	40(19.05)	210
15	Clindamycin	165(78.57)	24(11.43)	21(10)	210
16	Chloramphenicol	162(77.14)	14(6.67)	34(16.19)	210
17	Cefotaxime	139(66.19)	37(17.16)	34(16.19)	210
18	Penicillin	168(80)	25(11.90)	17(8.09)	210
19	Tetracycline	208(99.04)	2(0.95)	0	210
20	Erythromycin	165(78.57)	25(11.90)	20(9.52)	210
21	Oxacillin/Cephoxitin	197(93.81)	2(0.95)	11(5.24)	210

DISCUSSION

The study was undertaken to determine the incidence of urinary tract infection in various age group to evaluate the bacterial agents involved in this UTI, as well as their sensitivity as well as resistance pattern towards various antimicrobial agents so that there can be some improvement in efficient empirical treatment for the much effective cure of UTI.

Increasing antimicrobial resistance has been documented globally⁷⁻¹³. The prevalence of UTI was found to be 65.28% in this study and which accounts for 53.82%¹⁴, 49.11%¹⁵ 38.6%¹⁶, 35.5%¹⁷, 34.5%¹⁸, and 36.68%¹⁹ in India; however, the prevalence rate of UTI in our study correlates with other studies done in South Trinidad²⁰, and in the Mexican population²¹ which showed such more highly significant uropathogens 49% and 97.3%, respectively.

Our study showed a high prevalence of UTI in females (69.34%) than in males (30.66%) which correlates with other findings which revealed that the frequency of UTI is greater in females as compared to males^{14,15,22,10,20,21,23-25}. The reason behind this high prevalence of UTI in females is due to close proximity of the urethral meatus to the anus, shorter urethra, sexual intercourse, incontinence, and bad toilet²⁶⁻²⁸.

Females of the age group 31-40 years were found more susceptible (40.48%) to UTI followed by 41-50 years (23.81%), 20-30 years (16.67%), 51-60 years (11.90%) and ≥60 years (7.14%). These findings correlate with other reports which showed that females are more prone to UTIs than males during adolescence and adulthood^{25,38,39}. The factors of this increasing incidence of UTI in young age females are associated with high sexual activity, unhygienic conditions and a history of recurrent UTIs²⁹.

Escherichia coli (26.19%) was found the most prevalent gram negative bacteria in the positive urine samples of UTI. This result is consistent with reports from other studies^{18,30,31,32,33-35} but differs from the reports in which *P. aeruginosa*³⁶ and *Klebsiella* spp.³⁷ were bacteria from UTI cases. In this study the second common uropathogens isolated was *K. pneumoniae* (10.95%), *P. aeruginosa* (6.67%), *Enterococcus* spp (5.34%), *S. aureus* (4.76%), *C. albicans* (2.38%). These findings were not correlate with other reports in which *P. aeruginosa* was reported as the second most common bacterial isolate in UTI studies in India³⁸ and Lafia, Nigeria³⁹; however, these results correlates with others in which *Klebsiella* spp. was reported as the second most frequently isolated

organism in UTI^{12,35,40-42}. Studies on UTI conducted in other places of the world concluded that *E. coli* and *Klebsiella* spp. are the commonest uropathogens in UTI⁴³⁻⁴⁷.

E. coli is the major uropathogens, causing UTI, has many factors which are responsible for their attachment to the uroepithelium. In addition, they are able to colonize in the urogenital mucosa with adhesins, pili, fimbriae, and P-1 blood group phenotype receptor⁴⁸. In females of all age categories, *E. coli* is the most frequently isolated uropathogen which correlates with other studies⁴⁹⁻⁵¹ but not with others which found that *E. coli* causes most male UTIs, followed by other Enterobacteriaceae frequently isolated in the younger female patients of UTI and *K. pneumoniae* in the elderly patients⁵⁰.

Empiric antibiotic therapy is the mainstay of treatment for UTIs. However, many previous studies indicate increasing trend of drug resistance among UTI pathogens.

Table 3, in our study, shows the sensitivity as well as resistance pattern of isolated uropathogens towards antimicrobial agents as far as sensitivity is concerned it was observed as - The sensitivity was observed as 46.19% to Amikacin, 42.86% to Norfloxacin, 42.38% to Nitrofurantoin 40.48% to Gentamicin, 30.95% to Meropenem, 25.71% to Ciprofloxacin, 20.95% to Piperacillin-tazobactam and 19.05% to Linezolid, 18.57% to Co-trimoxazole, 17.14% to Amoxicillin-clavulanic acid, 16.19% to Chloramphenicol and Cefotaxime, 13.81% to Vancomycin, 12.38% to Co-trimoxazole.

On the other hand, as far as the resistance of uropathogens towards antibiotics is concerned, it is found as - 29.05% to Ciprofloxacin, 22.38% to Ampicillin, 21.43% to Amoxicillin-clavulanic acid, 20.47% to Co-trimoxazole, 17.61% to Cefotaxime, 12.86% cefipime and 11.90% to Erythromycin and Penicillin, 11.43% Clindamycin, 10.95% Norfloxacin, 10% Gentamicin, 9.04% Amikacin. Resistance towards other pathogens contributes as 7.14%, 6.67%, 5.71%, 4.75%, 3.81%, 3.33%.

The uropathogens were most sensitive towards amikacin(46.19%) followed by Norfloxacin(42.86%) and nitrofurantoin (40.48%) were as resistance towards these antimicrobial agents 9.04% Amikacin, 10.95% Norfloxacin, 7.14% nitrofurantoin.

From the results we can see that, *E. coli* and *K. pneumoniae* are main the etiological agents, norfloxacin, nitrofurantoin can be judiciously used as a first line antibiotic in treating community acquired UTIs. Most of the isolates were resistant to third and fourth generation cephalosporins and the commonly used antibiotic β-lactam and β-lactamase inhibitor combinations, which indicates the inappropriate and injudicious use of these antibiotics as empirical therapy. Hence necessitates the need for an antibiotic policy in every hospital.

CONCLUSION

This study determined the incidence of urinary tract infection and highlighted the major bacterial agent causing UTI. The pattern of isolates and antibiotics reported in this study is consistent with the usually reported pattern, with *Escherichia coli* being the most common organism isolated in cases of urinary tract infection. The high rate of resistance to Ciprofloxacin, Ampicillin, Amoxicillin-clavulanic acid, Co-trimoxazole, Cefuroxime, Cefazolin, Cefotaxime, of urinary isolates, precludes the use of these commonly used antibiotics for empiric treatment of UTI in India, where as sensitivity towards amikacin, norfloxacin, nitrofurantoin recommends there use for the treatment.

Therefore, urine culture is necessarily recommended for screening and diagnosis.

Since urinary tract infection may be asymptomatic in most cases (as this study has shown), it is therefore suggested that routine screening of patients of PUO (Pyrexia of unknown origin) should be done for urinary tract infection and the appropriate antimicrobials administered after antibiotic sensitivity tests have been carried out in order to prevent the cases becoming symptomatic later with resultant renal damage.

Routine monitoring of resistance as well sensitivity patterns is necessary, which will help the clinicians in the empirical treatment of UTI and also for preparation of an antibiotic policy.

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