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Research Article

UROPATHOGENS AND THEIR RESISTANCE PATTERN IN URINARY TRACT INFECTION CASES OF TERTIARY CARE HOSPITAL, BHOPAL

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ABSTRACT

Objectives: Urinary tract infections (UTIs) are an increasing public health problem caused by various uropathogens. To assess the adequacy of empirical therapy, an antibiogram of the bacteria responsible for UTI in patients coming to the tertiary hospital, Bhopal was evaluated for 13 months.

Methods: In this prospective and observational study, all urine samples from patients of a tertiary care hospital collected at the Department of Microbiology, Peoples College of Medical Sciences and Research Centre, Bhopal, from January 2014 to January 2015 were processed. A sample with more than 10^5 CFU/mL of bacteria was considered positive, the bacteria were identified, and antibiotic susceptibility profile was characterized.

Results: A total of 283 urine samples from suspected UTIs were analyzed, for which identification of bacteria and antimicrobial susceptibility testing were done. Overall, 56.53% were culture positive with a predominance of female patients (70.62%). Females 26–35 years old and males ≥46 years old showed maximum culture positivity. *Escherichia coli* (55.7%) was the most commonly isolated microorganism, followed by *Klebsiella pneumoniae* (24.8%). Isolated uropathogens were predominantly resistant to Ampicillin, Amoxycillin clavulanic acid, Cefotaxime, Ceftazidime, Cotrimoxazole, and Nalidixic acid.

Conclusion: Our study confirms a global trend toward increased resistance to most antibiotics. We emphasize the formulation of antibiotic policy for a particular geographical area. *E. coli* was the most common uropathogen. Nitrofurantoin, Fluoroquinolones, Amikacin, and Piperacillin/tazobactam were the most effective antibiotics against uropathogens.

Keywords: *Escherichia coli, Klebsiella pneumoniae*, Urinary tract infection, Antibiotic resistance, Uropathogens.

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INTRODUCTION

Urinary tract infections (UTIs) are the second most common infectious disease in a community and accounts significant cause of morbidity too. Approximately 150 million cases of UTIs are diagnosed each year [1]. Bacteriuria with urinary symptoms is known as UTI, and it is divided into two types: Simple UTI and complicated UTI. Uncomplicated UTIs are common in healthy adults. Persons with renal disorders such as obstructions, calculi, catheterization, and transplantation are more prone to complicated UTIs.

Because female urethra is less effective at preventing bacterial entry, UTI is more common in women [2]. UTI is commonly seen in females because of bacterial colonization in the vagina, sexual activity, and pregnancy [3]. UTI is caused by bacteria, fungi, parasites, and viruses, but bacteria alone accounts for more than 95%.

UTI is commonly caused by Gram-negative bacteria, such as *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Proteus* species. Among Gram-negative, 75–95% of cases of UTI are caused by *E. coli*. Gram-positive bacteria, *Staphylococcus aureus*, *Staphylococcus saprophyticus, and Enterococcus* species are most commonly responsible for UTIs [4].

Broad-spectrum antibiotics have been very effective in the management of UTIs; therefore, they are commonly prescribed. Today's scenario is that inappropriate use and over-the-counter availability of antibiotics have led to the global emergence of antibiotic resistance against uropathogens [5].

Frequently changing antimicrobial susceptibility patterns and the emergence of multidrug-resistant (MDR) bacterial pathogens are the leading cause of morbidity and mortality. Microbial isolation in UTIs and their antibiotic susceptibility is crucial before starting empirical treatment and also for the prevention of the emergence of antimicrobial resistance.

Few studies have been reported from Madhya Pradesh on uropathogenic antibio grams. The purpose of this study was to identify common uropathogens and their resistance patterns in suspected UTI cases attending a tertiary care hospital in Bhopal.

METHODS

Study design

It is an observational and prospective study carried out in the Department of Microbiology, People's College of Medical Sciences and Research Centre, Bhopal, Madhya Pradesh, from January 2014 to January 2015 for 13 months after approval of the Ethical committee. Two hundred and eighty-three urine samples from patients suspected of UTI of all age groups and both gender willing to participate were included in the study.

Sample collection and transport

Early morning mid-stream urine specimen collected in a sterile, dry, and wide-mouth leak pro of labeled container was instantly transported to the microbiology laboratory at People's College of Medical Sciences and Research Centre, Bhopal, for further processing.

Processing of sample

A urine sample was inoculated on cysteine lactose electrolytes deficient agar by using a calibrated wire loop (0.001 mL) and overnight incubated at 37°C. Samples showing Colony counts >10^5 CFU/mL were further processed for the identification of bacterial species using standard microbiological techniques, such as Gram staining, colony morphology, and biochemical testing [6].

The antibiotic susceptibility testing was done by the Kirby Bauer disc diffusion method on Müller–Hinton agar as per CLSI 2014 recommendations. For Gram-negative *E. coli* (ATCC 25922) and for Gram-positive *S. aureus* (ATCC 29213) were used for quality control. Antibiotic discs procured from HiMedia and its concentrations (µg) used for Gram-negative bacteria were as follows: Amikacin (30), Ampicillin (10), Amoxicillinclavulanic acid (30), Ceftazidime (30), Cefotaxime (30), Ciprofloxacin (5), Cotrimoxazole: trimethoprim-sulphamethoxazole (25), Gentamicin (10), Imipenem (10), Meropenem (10), Nalidixic acid (30), Nitrofurantoin (30), Norfloxacin (10),Ofloxacin (5), and tazocin: Piperacillin-tazobactam (40), The antibiotic discs and concentrations (µg) used for Gram-positive bacteria were as follows Cefoxitin (30), Cotrimoxazole: trimethoprim-sulphamethoxazole (25), Ciprofloxacillin (5), Gentamicin (10), Linezolid (10), Nitrofurantoin (30), Norfloxacin (10), Vancomycin (30), and Teicoplanin (30). The zone of inhibition of antibiotics was measured and interpreted according to the CLSI 2014 guidelines [7].

Statistical analysis

Data were collected in Microsoft Excel and result was analyzed and expressed in percentages and Pearson Chi-square test.

RESULTS

A total of 283 urine specimens from outpatients and inpatients were received during the study period, out of which 140 samples (49.46%) were from male patients and 143 samples (50.53%) were from female patients showing symptoms of UTI. Out of the total sample received, 160 were culture-positive. Hence, the overall prevalence of UTI was 56.53%. In this study, most of the uropathogens were recovered from female patients (70.62%) and male patients (42.14%), as shown in Table 1. Various predisposing factors contribute to the higher prevalence of UTIs among women.

Culture proven UTI patients showed Chi-square test statistically significant (p<0.05) at a 95% level of the confidence interval. The Chi-square test values were χ^2=23.3617; degree of freedom=1; p<0.00001. The result was significant at p<0.05.

As per Fig. 1, overall highest susceptible age group of patients to UTI was ≥46 years (72%). An increasing trend was seen from 36 to 45 years (42.2%), then 26–35 years (53.8%) and 15–25 years (57.6%).

Comparative analysis of UTI in both genders in all age groups (Tables 2 and 3) shows a higher prevalence of UTI among females than in males in 15–45 age groups, but in ≥46 years age group, the preponderance was seen in males.

In females, age group of 26–35 years had an 87.8% prevalence of UTI; however, in males, the highest susceptible age group to UTI was ≥46 years (86.6%).

Variables for infected males and non-infected males the Pearson Chi-square test values were as follows: 2=57.0002; degree of freedom=1; p<0.00001. As per Table 2, the result was significant at p<0.05.

For the infected and not infected female patient's variable, the Chi-square test values were χ^2=18.553; degree of freedom=1; p≤0.000338. As per Table 3, the result was significant at p<0.05.

Table 4 shows the highest female to male UTI ratio in the age group of 15–25 years (16:1), followed by 26–36 years (6:1), 36–45 years (1.5:1), and ≥46 years (0.38:1).

Female to male UTI trend differ significantly (p<0.05) with χ^2=52.3479; degree of freedom=1; p<0.00001.

Table 1: Culture proved among UTI patients

Gender	Positive % (n)	Negative % (n)	X^2	p-value
Male	42.14 (59)	57.85% (81)	23.3617	<0.00001*
Female	70.62 (101)	29.37% (42)		
Total	56.53 (160)	43.46% (123)		

*Values are statistically significant by Pearson Chi-square test; *P*<0.05.
UTI: Urinary tract infection

Table 2: Prevalence of UTI among the different age groups of male UTI patients

Age group	Total male	Infected male (%)	Not infected male (%)	X^2	p-value
15–25 years	20	2 (10)	18 (90)	57.0002	<0.00001*
26–35 years	37	6 (16.2)	31 (83.7)		
36–45 years	38	12 (31.6)	26 (68.4)		
≥46 years	45	39 (86.7)	6 (13.3)		
Total	140	59 (42.14)	81 (57.8)		

*Values are statistically significant by Pearson Chi-square test; *P*<0.05.
UTI: Urinary tract infection

Table 3: Prevalence of UTI among the different age groups of female UTI patients

Age group	Total female	Infected female (%)	Not infected female (%)	Pearson Chi - square test X^2	p-value
15–25 years	39	32 (82)	7 (17.9)	18.553	<0.000338*
26–35 years	41	36 (87.8)	5 (12.1)		
36–45 years	33	18 (54.5)	15 (45.4)		
≥46 years	30	15 (50)	15 (50)		
Total	143	101 (70.62)	42 (29.3)		

*Values are statistically significant by Pearson Chi-square test; *P*<0.05.
UTI: Urinary tract infection

Table 4: Female-male ratio in UTI patients

Age group	Infected female (n)	Infected male (n)	Infected female male ratio
15–25 years	32	2	16:1
26–35 years	36	6	6:1
36–45 years	18	12	1.5:1
≥46 years	15	39	0.38:1

UTI: Urinary tract infection

As per Fig. 2, a total of 160 bacterial uropathogens comprised 149 (93.1%) Gram-negative bacteria and 11 (6.8%) Gram-positive bacteria isolated from culture-positive urine samples.

According to Fig. 3, dominant bacteria among 149Gram-negativeuropathogens *E. coli* were found to be 55.7%, followed by *K. pneumoniae* 24.8%. Other organisms, such as *P. aeruginosa and Proteus vulgar,* are represented 11.4% and 8%, respectively.

S. aureus (72.7%) was the most common Gram-positive isolates followed by coagulase negative *Staphylococcus* (CONS) (18%) and *Enterococcus* species (9%).

The prevalence of the most dominant bacteria *E. coli* as per Table 5 shows 41.9% in females and 16.4% in males. The Pearson Chi-square

Table 5: Comparison of demographic variables with the prevalence of *E. coli.* (n=83)

Variables	Category	*E. coli. present*	*E. coli. absent*	Prevalence (%)	X2	p-value
Gender	Female	60	83	41.9	4.76	0.029
	Male	23	117	16.4		
Age groups	15–25 years	19	40	32.2	14.94	0.002
	26–35 years	32	46	41.0		
	36–45 years	9	62	12.6		
	≥46 years	23	52	30.6		

*Values are statistically significant by Pearson Chi-square test; *P*<0.05. *E. coli: Escherichia coli*

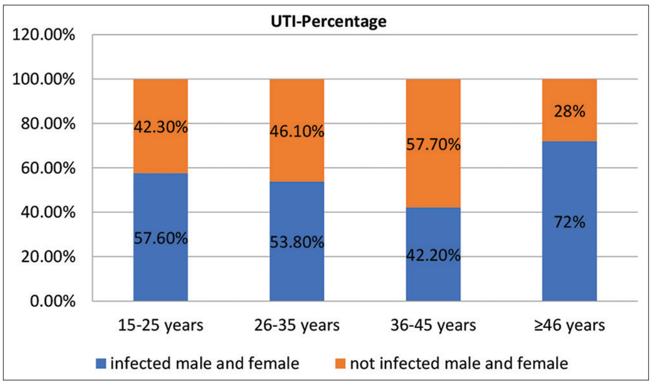

Fig. 1: Prevalence of UTI among the different age groups in culture-positive samples

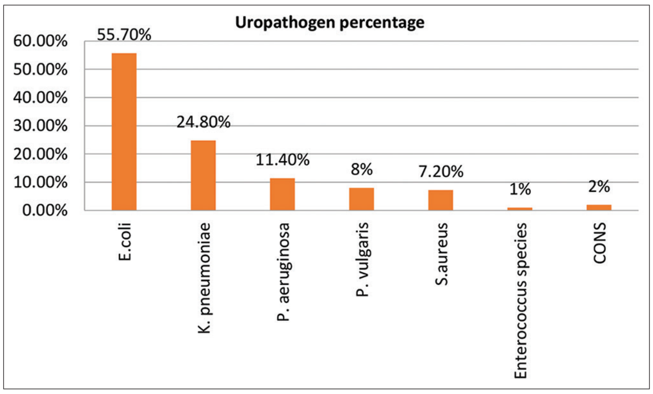

Fig. 3: Percentage of uropathogens detected in culture-positive samples

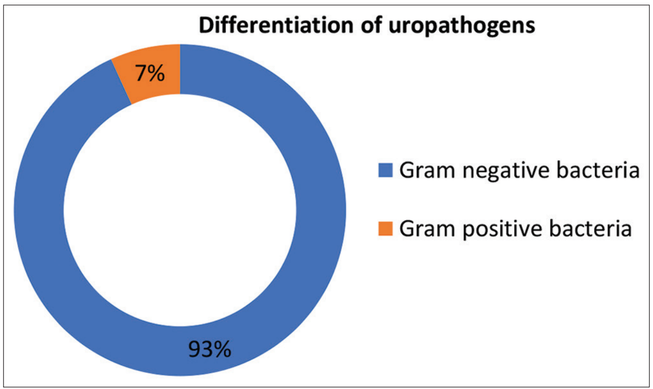

Fig. 2: Uropathogens in culture-positive urinary tract infections patients (n=160)

test results were χ^2=4.76; degree of freedom=1; p<0.029. The outcome was significant at p<0.05.

In overall UTI patients aged 26–35 years showed a maximum growth of 41% of *E. coli.* The Pearson Chi-square test values were χ^2=14.94; degree of freedom=1; p<0.002. The result was significant at p<0.05.

Antibiotic resistance of isolated Gram-negative uropathogens shown in Table 6 has the highest percentages of resistance against most of the tested antibiotics, with more than 50% resistance to broad-spectrum antibiotics. However, a low level of resistance has been observed for Gram-negative agents against Carbapenems.

In our study, the highest resistance was observed against *E. coli* to almost all the tested antibiotics which were in the range of 10–60%, except Carbapenems.

K. pneumoniae showed nearly 30% resistance to third-generation cephalosporins. The observed resistance for *K. pneumoniae* was 8% against Amikacin whereas Carbapenems were found to be 100% sensitive.

P. aeruginosa showed nearly 50% resistance to Ciprofloxacin and Ceftazidime. All *P. aeruginosa* isolates were sensitive to Imipenem and Meropenem.

Proteus vulgaris was resistant to 15–25% cephalosporine and aminoglycoside tested.

This massive increase in antibiotic resistance is a result of the overuse of these antibiotics for the treatment of different infections in our region without checking culture sensitivity. This alarming situation is the leading cause of MDR infection among UTIs.

According to Table 6, Gram-positive isolates were completely sensitivity to Linezolid, Teicoplanin, and Vancomycin.

S. aureus showed 62.5% resistance to Cefoxitin and Cotrimoxazole and 50% to Norfloxacin. Nitrofurantoin turned out to be effective with *S. aureus* as it showed 12.5% resistance only.

CONS is resistant to Norfloxacin and Cotrimoxazole by 50%.

Enterococcus species were sensitive to all tested antibiotics.

DISCUSSION

Correct bacterial isolate identification and antibiotic selection assist clinicians in the effective management of bacterial UTI. Our study describes the distribution and antibiotic resistance pattern of microbial species isolated from populations with suspected UTIs. During the study period, 283 urine samples from different age groups and both genders were analyzed, out of which 160 (56.53%) were culture positive, which is at par with other studies. Our findings are also in agreement with this generalization and rightly coincide with a study done by Waske *et al.* [8] and Prakash and Saxena *et al.* [9]. This study gives an insight into UTIs, one of the most common infections leading to an antibiotic prescription from a tertiary care hospital. About 70.62% of female patients were culture positive, compared to 42.14%, of male patients, indicating UTI is more common in females as revealed in other studies [10]. Long urethra and bacteriostatic prostatic secretions are the cause of low percentage of UTI in males [11].

Table 6: Antibiotic resistance pattern of uropathogens

Antibiotics	*Escherichia coli* % n=83	*Klebsiella pneumoniae* % n=37	*Pseudomonas aeruginosa* % n=17	*Proteus vulgaris* % n=12	*Staphylococcus aureus* % n=8	*Enterococcus* species % n=1	CONS % n=2
Amikacin	5 (6)	3 (8)	3 (17.6)	2 (16.6)	-	-	-
Ampicillin	47 (56.6)	15 (40.5)	-	-	-	-	-
Amoxycillin clavulanic acid	45 (54.2)	11 (29.7)	-	-	-	-	-
Cefotaxime	47 (56.6)	11 (29.7)	-	2 (16.6)	-	-	-
Cefoxitin	-	-	-	-	5 (62.5)	0 (0)	0 (0)
Ceftazidime	45 (54.2)	10 (27)	8 (47)	2 (16.6)	-	-	-
Ciprofloxacin	5 (6)	-	9 (52.9)	0 (0)	3 (37.5)	0 (0)	0 (0)
Cotrimoxazole	46 (55.4)	12 (32.4)	-	-	5 (62.5)	0 (0)	1 (50)
Gentamicin	37 (44.5)	9 (24.3)	5 (29.4)	3 (25)	-	-	-
Linezolid	-	-	-	-	0 (0)	0 (0)	0 (0)
Imipenem	2 (2)	0 (0)	0 (0)	0 (0)	-	-	-
Meropenem	2 (2)	0 (0)	0 (0)	0 (0)	-	-	-
Norfloxacin	8 (9.6)	3 (8)	1 (5.8)	0 (0)	4 (50)	0 (0)	0 (0)
Nitrofurantoin	7 (8.4)	3 (8)	1 (5.8)	-	1 (12.5)	0 (0)	1 (50)
Nalidixic acid	50 (60.2)	7 (18.9)	2 (11.7)	-	-	-	-
Piperacillin tazobactum	5 (6)	0 (0)	5 (29.4)	0 (0)	-	-	-
Teicoplanin	-	-	-	-	0 (0)	0 (0)	0 (0)
Vancomycin	-	-	-	-	0 (0)	0 (0)	0 (0)

In our study, the majority (72%) of cases were older than 46 years old, compared to young patients (15–25 years, 57.60%; 26–35 years, 53.80%) and middle-aged patients (36–45 years, 42.2%) which differs from the other studies. Devki *et al.* in West Bengal found the highest incidence of UTI among the 20–40 years (55.62%.) age group [12].

UTIs are common in women, and often associated with significant morbidity and mortality. Various predisposing factors contribute to the higher prevalence of UTIs among women and may affect women of all age groups, especially sexually active ones [13]. This could be because of the highest prevalence rate of 87.8% observed in the 26–35 years age group. It is followed by 15–25 years (82%), then 36–45 years (54.5%), and lowest among the ≥46 years age (50%). UTI is more common in females than males during adolescence and adulthood because of high sexual activity [14].

Males above 46 years showed an 86.6% incidence of UTI which is similar to the study by Smita *et al.* [15]. In male, prostate enlargement and neurogenic bladder seen with advancing age are major causes of increased incidence of UTI [16].

The female-male ratio of UTI was seen in a decreasing trend in the age group of 15–25 years (16: 1), followed by 26–35 years (6: 1), 36–45 years (1.5: 1), and ≥46 years (0.38: 1).

In our study, Gram-negative bacilli constituted 93.1% of the total bacterial isolates, while Gram-positive cocci constituted 6.8%. Isolated uropathogens were *E. coli* followed by *K. pneumonia* which is similar to many other studies from India, such as Majumder *et al*., 2018 [17].

E. coli (55.7%) was the most prevalent bacteria involved in UTIs, which is similar to the other studies with a range between 40% and 78%. Second most common uropathogen isolated was *K. pneumoniae* (24.8%) which was much higher than other studies with a range between 11% and 17% [18,19].

Moreover, organisms such as *Pseudomonas* spp., *Proteus* spp. *S. aureus*, CONS, *and Enterococcus* spp. represented 11.4%, 8%, 7.2%, 2%, and 1%, respectively. The highest prevalence of Gram-negative organisms was seen in our study. The scale of bacterial uropathogens, on other hand, varies with topographical location and characterization of the patients UTIs

Empirical use and overuse of antibiotics in UTIs are responsible for antibiotic resistance. Due to increasing resistance, it has been difficult for the clinician to treat UTIs as they are left with only a few drugs. Our study revealed that carbapenem was highly active against members of *Enterobacteriaceae* followed by Amikacin and Piperacillin/tazobactam. Similar findings were drawn by Rakesh *et al.* [20] and Deshpande *et al.* [21].

The antimicrobial resistance patterns of *E. coli.* showed a high frequency of resistance ranging from 60 to 45% to most of the drugs which included Nalidixic acid (60.2%), Ampicillin and Cefotaxime (56.6%), Cotrimoxazole (55.4%), Amoxyclavulanic acid and Ceftazidime (54.2%), and Gentamycin (44.5%) whereas low resistance was seen by Norfloxacin (9.6%), Nitrofurantoin (8.4%), Ciprofloxacin, Piperacillin tazobactam and Amikacin (6%), and Imipenem and Meropenem (2%).

K. pneumoniae showed a high frequency of resistance to Ampicillin (40.5%), Cotrimoxazole (32.4%), Cefotaxime (29.7%), Ceftazidime (27%), Gentamicin (24.3%) and Nalidixic acid (18.9%), and Nitrofurantoin, Amikacin, and Norfloxacin (8%). Meropenem, Imipenem, and Piperacillin tazobactam were all highly sensitive for this organism.

P. vulgaris showed 25% resistance to Gentamicin and 16.6% resistance to Amikacin, Cefotaxime, and Ceftazidime. Imipenem and Meropenem showed 100% sensitivity.

P. aeruginosa has shown 100% susceptibility to Imipenem and Meropenem which is at par with Deshpande *et al.* 2011 [17]. In our study, resistance to Piperacillin Tazobactam by *P. aeruginosa* was found to be nearly 30% which coincides with that of Baveja *et al.* 2014 [22].

Easily availability of antibiotics might be responsible for antibiotic resistance. Physicians prescribing antibiotics without laboratory confirmation may be one of the causes of increasing resistance in UTI isolates. The study by McEwen *et al.* found that physicians prescribe Trimethoprim-sulphamethoxazole 37% and Fluoroquinolones 32% [23].

In our study, from all the Gram-positive cocci isolated, 100% were susceptible to Vancomycin, Linezolid, and Teicoplanin which was at par with Rakesh *et al.* 2014 [20].

It is a common trend to treat uncomplicated UTIs with a short course of empirical oral antibiotics. Microbiological evaluation of UTI is done only in complicated UTIs. Overall, Gram-negative isolates showed a higher resistance pattern in comparison to Gram-positive isolates in the present study.

CONCLUSION

Microbiological evaluation of all symptomatic UTIs should be made mandatory; no empirical antibiotic treatment should be done. Constant monitoring of the culture and sensitivity patterns of specific pathogens is required in our country. There is an urgent need for community awareness regarding the emergence of MDR strains and their preventive measures. More and more studies are required on uropathogenic resistance patterns in our region. Our study is a contribution to reporting the culture and sensitivity patterns of the bacterial isolates in symptomatic UTI cases visiting tertiary hospitals.

AUTHORS CONTRIBUTION

Surender Kaur: The writer collected the data, structured it and designed the analysis. Abhijit Awari: He contributed in analyzing and helped in structuring and editing the paper.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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