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Original Research Article.

Incidence and Antibiotic Resistance Pattern of Uropathogens Isolated from Suspected Cases of Urinary Tract Infection

Abhishek Gaur[1], Rakesh Kumar[2], Prashant Panda[3], Mihir Kumar Pattanayak[4*]

[1]Associate Professor, [2]Senior Resident, [3]Demonstrator, [4*]Assistant Professor,
Department of Microbiology, LSLAM Government Medical College, Raigarh, Chattisgarh, India.

ABSTRACT

Background: Urinary tract infections are most common and frequently occur in both the community and hospital settings as well. The complications of urinary tract infections causing longer hospital stay, increased expenses in patient care and mortality.

Aim: Aetiology of UTI and their antibiotic resistance patterns may vary from time to time and place to place. This retrospective study was conducted in Department of Microbiology, GMC, Raigarh, CG, from January 2015 to December 2015 with the aim to know about the uropathogens and their antibiotic susceptibility pattern of this region.

Methods and Results: All suspected cases of urinary tract infection (UTI) were included in the study those who attended the hospital during the period from January 2015 to December 2015. A total of 278 urine specimens were collected from patients suspected of having UTI, out of which only 115 urine samples showed significant growth with 8 Candida spp. and the rests were bacteria i.e., 107 grown. The most common etiological organisms isolated were *Escherichia coli* (32.8%), *K. pneumoniae* (18.2%), *K. oxytoca* (4.6%), *P. aeruginosa* (7.4%),*A. baumanii* (5.6%), *Morganella morganii* (1.8%), *Citrobacter* spp. (5.6%) and *Enterobacter* spp. (2.8%) however the gram positive bacteria includes *Staphylococcus aureus* (10.2%), Coagulase negative *Staphylococci* (CoNS) (5.6%) and *Enterococcus* spp. (3.7%) isolated at low percentage. Antibiogram study shows, most of the gram negative bacilli found resistance to aminopenicillins less often fluoroquinolones but shows more sensitivity to aminoglycosides group of antibiotics, whereas gram positive cocci shows maximum sensitivity to glycopeptide group of antibiotics.

Conclusion: Present study shows most of the organisms isolated from urine samples shown resistance to multiple drugs which is of great concern. A strict antibiotic policy and infection control practice is the need of the hour.

Key words: Urinary Tract Infection, Antibiotic Resistance, Significant Growth, Bacteriuria.

***Correspondence to:**

Mihir Kumar Pattanayak,
Assistant Professor,
Department of Microbiology,
LSLAM GMC, Raigarh, Chattisgarh, India.

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INTRODUCTION

Urinary tract infection (UTI) is the most common and serious health affecting problem not only in hospital settings but in the community as well. UTIs are much more common in elderly than younger individuals for a variety of reasons but can infect any age group. It is the second most common infection after respiratory tract infection commonly encountered diseases in developing countries with an estimated annual global incidence of at least 250 million. It is the most important cause of morbidity globally affecting all age groups at least once in their life span in both genders and usually requires medical treatment.[1]

A urinary tract infection (UTI) involving infections in kidney, ureters, bladder or urethra which are the part of the urinary tract. Symptoms from a lower urinary tract include painful urination, frequent urination or urge to urinate (or both). In the elderly and the very young, symptoms may be vague or non-specific.

UTIs refer to the presence of microbial pathogens within the urinary tract and it is usually classified by the infection site; bladder (cystitis), kidney (pyelonephritis), or urine (bacteriuria). UTIs that occur in a normal genitourinary tract with no prior instrumentation are considered as “uncomplicated,” whereas “complicated” infections are diagnosed in genitourinary tracts that have structural or functional abnormalities, including instrumentation such as indwelling urethral catheters, and are frequently asymptomatic. Many different microorganisms can cause UTIs though the most common pathogeninvolve with in the community is *Escherichia coli* and rest are other Enterobacteriaceae, which accounts approximately 75% of the total isolates. In complicated urinary tract infections in hospitalized patients, organisms such as *Enterococcus spp.*, multi drug resistant gram - negative rods including *Pseudomonas* spp. are

more common. The relative frequency of the pathogens varies depending upon age, sex, catheterization, and hospitalization. The emergence of antibiotic resistance in the management of UTI is a serious public health issue, particularly in the developing world where majority of populations living below poverty line, ignorance and poor hygienic practices, there is also a high prevalence of fake and low quality drugs in circulation.[2] In patients with suspected UTI, antibiotic treatment is usually started empirically though the appropriate treatment should initiate only after isolation of the organisms and its susceptibility pattern.[3] Hence the changing spectrum of microorganisms involved in UTI and emergence of resistance across institutions and geographical areas have made imperative the conduct of antibiotic susceptibility pattern study of UTI pathogens in various regions from time to time.[4] Taking to the note and knowing about the organisms isolated from UTIs and their susceptibility pattern of this region, the present study was conducted to isolate and identify the uropathogens from suspected UTI patients who attended the hospital (either OPD or IPD) and further study their antibiotic susceptibility pattern.

MATERIALS AND METHODS

This observational and prospective study was conducted at Govt. Medical College, Raigarh, Chhattisgarh from January 2015 to December 2015. A total 278 clean catch midstream urine samples were collected in a wide mouth sterile container from inpatients (IPD) and out patients (OPD) who are not on antimicrobials.

BACTERIOLOGY

Isolation of uropathogens was performed by a surface streak procedure on both blood and cysteine lactose electrolyte deficient (CLED) medium using calibrated loops for semi-quantitative method and incubated aerobically at 37°C for overnight, and those samples which became negative after overnight incubation were further re-incubated for 24 hours. A specimen was considered positive for UTI if a single organism was isolated at a concentration of $\geq 10^5$cfu/ml.[5] Out of the 278 urine samples, 107 bacterial isolates plus 8 Candida spp. were recovered and conferred to Kass concept of significant bacteriuria were further identified and evaluated for their antibiotic susceptibility using Kirby-Bauer methods.[5,6] Bacterial identification was made using panel of biochemical tests, namely indole, bile-esculin test, citrate, oxidase, H_2S production, sugar fermentation, lactose fermentation, urea hydrolysis, gas production, catalase, coagulase, mannitol fermentation and novobiocin susceptibility test.

ANTIBIOTIC SUSCEPTIBILITY TESTING

The antibacterial susceptibility testing of the isolates was done using the Kirby-Bauer disk diffusion method[6] in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2006) guidelines using antibiotics containing discs from Oxoid. Briefly, 20 ml of Mueller- Hinton agar (Difco Laboratories GmbH, Augsburg, Germany) was prepared and poured into sterile plates. A standard inoculum adjusted to 0.5 McFarland was swabbed on to Muller – Hinton agar (Oxoid Ltd. Bashingstore Hampaire, UK) and the medium was allowed to solidify at room temperature. Further few colonies of an 18 h culture of the isolates were streaked on the surfaces of the dried Muller-Hinton agar plates. Then the selected antibiotic discs were gently and firmly placed on the agar plates, which were then left at room temperature for 1 hour to allow diffusion of the antibiotics into the agar medium. The plates were then incubated at 35 - 37°C for 24 hours. Zones of bacterial growth inhibition were then measured to the nearest millimetre scale and interpreted as per CLSI guidelines. The antibiotic discs and their concentrations were: ampicillin 10 mcg, amoxicillin 20mcg, amoxicillin/clavulanic acid 30 mcg, ciprofloxacin 5 mcg, ofloxacin 5 mcg, co-trimoxazole 25 mcg, ceftazidime 10 mcg, ceftazidime/clavulanic acid 30/10 mcg, clindamycin 2 mcg, erythromycin15 mcg, gentamicin 30 mcg, imipenem 10 mcg, nitrofurantoin 300 mcg, nalidixic acid 30mcg, linezolid 15 mcg, piperacillin/tazobactum 100/10mcg, amikacin 30 mcg, netilin 30 mcg, vancomycin 5 mcg, tigecycline 15 mcg. The reference strains used as control were *E.coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853).

Table 01: Number of Organism Isolated

Gram Positive Cocci	Gram Negative Bacilli	Candida spp.
21	86	8

Table 02: UTI cases in Male and Female

UTI cases (N=115)	Male	Female
	38	77

Table 03: Uropathogens isolated at various age groups of male and female

AGE GROUPS (YEARS)	MALE (n=38)	FEMALE .(n=77)
10-19	4 (10.5%)	6 (7.8%)
20-29	9 (23.6%)	42 (54.5%)
30-39	5 (13.1%)	14 (18.1%)
40-49	1 (2.7%)	5 (6.5%)
50-59	10 (26.3%)	4 (5.2%)
60-80	9 (23.6%)	6 (7.8%)

RESULTS

Out of 278 urine specimens were collected from patients suspected of having UTI, out of which a total number of 115 (table 1) showed significant growth. A total of 08 candida species were isolated, so total bacteriuria is 107. Out of which 59 (55%) and 48 (45%) isolates (table 5) were recovered from inpatient and outpatient respectively. Among the total culture positive sample (115), 38 (33%) were male patients and 77 (67%) were female patients (table 2). The gram-negative bacteria constituted the largest group with 86 (80%) isolates while gram-positive bacteria constituted only 21 (20%) of the total isolates. Among the gram negative bacteria, following organisms recovered with percentage are: *E. coli* (32.8%), *K. pneumoniae* (18.2%), *K. oxytoca* (4.6%), *P. aeruginosa* (7.4%), *A. baumanii* (5.6%), *Morganella morganii* (1.8%), *Citrobacter* spp. (5.6%) and *Enterobacter* spp. (2.8%) however the gram positive bacteria includes *Staphylococcus aureus* (10.2%), Coagulase negative *Staphylococci* (CoNS) (5.6%) and *Enterococcus* spp. (3.7%).

The susceptibility of the clinical isolates to routinely prescribed antibiotics in the tertiary care hospital is depicted in table 7. *E. Coli* was the most prevalent bacteria and found susceptible to

ofloxacin, ciprofloxacin, gentamicin, amikacin, netillin, cefuroxime, ceftazidime, ceftazidime/clavulanic acid, cefipime, nalidixic acid, nitrofurantoin, pipercillin/tazobactam, imepenem, tigecycline.

Among these imepenem and tigecycline shows 100% susceptible but shows high degree of resistance to ampicillin and amoxicillin/clavulanic acid.

Table 04 : Isolated Uropathogens in Male and Female

Isolated Bacteria	Number	Male no.(%)	Female no.(%)
GRAM NEGATIVE BACILLI			
Escherichia coli	35	6 (17.2%)	29(82.8%)
Klebsiella pneumoniae	18	5 (27.7%)	13(72.3%)
Klebsiella oxytoca	5	2 (40%)	3(60%)
Pseudomonas aeruginosa	11	6 (54.5%)	5(45.5%)
Acinetobacter baumanii	6	3 (50%)	3(50%)
Morganella morganii	2	0	2(100%)
Citrobacter spp.	6	4 (66.6%)	2(33.4%)
Enterobacter spp	3	1 (33.3%)	2(66.7%)
GRAM POSITIVE COCCI			
Staphylococcus aureus	11	4(36.3%)	7 (63.7%)
Coagulase-negative staphylococci (CoNS)	6	2(33.3%)	4 (66.7%)
Enterococcus spp.	4	1(25%)	3 (75%)
Candida spp	8	4(50%)	4(50%)

Table 05: Uropathogens isolated from various outpatient and inpatient departments

Isolated bacteria(total no. 107)	Outpatient (total no.59)	Inpatient (total no.48)
GRAM NEGATIVE BACILLI (GNB)		
Escherichia coli (n=35)	24	11
Klebsiellapneumonia (n=18)	13	5
Klebsiellaoxytoca (n=5)	1	4
Pseudomonas aeruginosa (n=11)	3	8
Acinetobacerbaumanii (n=6)	1	5
Morganellamorganii (n=2)	1	1
Citrobacter spp. (n=6)	2	4
Enterobacter spp. (n=3)	1	2
GRAM POSITIVE COCCI		
Staphylococcus aureus (n=11)	8	3
Coagulase-negative staphylococci(CoNS) (n=6)	4	2
Enterococcus (n=4)	1	3

Table 06:Antibiotics Sensitivity pattern of gram positive cocci isolated from urine samples

Bacteria isolated	AMP	AMX	AMC	E	CD	G	CF	OF	COT	NIT	NA	VA	LZ
S. aureus (n=11)													
S	1	2	5	5	5	9	2	5	5	11	7	11	11
R	10	9	6	6	6	2	9	6	6	0	4	0	0
CoNS (n=6)													
S	2	2	3	2	2	4	1	2	4	6	4	6	6
R	4	4	3	4	4	2	5	4	2	0	2	0	0
Enterococcus spp. (n=4)													
S	0	2	3	0	0	3	2	1	1	4	2	4	4
R	4	2	1	4	4	1	2	3	3	0	2	0	0

Table 07:Antibiotic sensitivity pattern of gram negative bacilli isolated from urine samples

Bacteria isolated		Amp	Amc	Of	Cf	G	Ak	Nt	Cxm	Caz	CaC	Cpm	Na	Nit	Pit	I	Tgc
E. coli	S	3	7	11	11	28	34	34	26	30	34	30	32	32	28	35	35
(n=35)	R	32	28	24	24	7	1	1	9	5	1	5	3	3	7	0	0
K.pneumoniae	S	0	4	4	2	9	12	12	2	2	5	5	9	12	18	18	18
(n=18)	R	18	14	14	16	9	6	6	16	16	13	13	9	6	0	0	0
K. oxytoca	S	0	1	2	1	3	5	5	2	2	3	3	1	3	5	5	4
(n=5)	R	5	4	3	4	2	0	0	3	3	2	2	4	2	0	0	1
P. aeruginosa	S	0	2	4	2	5	10	10	4	6	8	3	5	8	8	11	9
(n=11)	R	11	9	7	9	6	1	1	7	5	3	8	6	3	3	0	2
A.baumanii	S	0	0	0	2	0	3	2	0	0	1	1	0	0	4	5	2
(n=6)	R	6	6	6	4	6	3	4	6	6	5	5	6	6	2	1	4
Citrobacter	S	2	2	1	2	2	3	3	2	2	2	2	1	3	6	6	4
spp. (n=6)	R	4	4	5	4	4	3	3	4	4	4	4	5	3	0	0	2
Enterobacter	S	0	1	1	3	3	3	3	3	3	3	3	3	3	3	3	3
spp (n=3)	R	3	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
M.morganii	S	0	2	2	1	1	2	2	2	2	2	2	2	2	2	2	2
(n=2)	R	2	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0

Amp=Ampicillin, Amx=Amoxicillin, Amc=Amoxicillin/clavulanic acid,Ak=Amikacin, Cd=Clindamycin, Caz= Ceftazidime, Cac = Ceftazidime/clavulanic acid,Cot=Cotrimoxazole, Cpm=Cefipime, Cf=Ciprofloxacin, Cxm=Cefuroxime, E= Erythromycin, G= Gentamicin, I= Imipenem, Lz= Linezolid, Na= Nalidixic acid, Nit= Nitrofurantoin, Nt= Netilin, Of= Ofloxacin, Pit= Pipercillin/tazobactam, Tgc=Tigecycline, Va= Vancomicin, R= Resistant, S= Sensitive

DISCUSSION

In community and hospital settings the etiology of UTIs and the antimicrobial susceptibility of UTI caused by bacteria have been changing over the years.[7,8] In this study, 59 (55%) culture positive urine sample collected from Out-door patient departments (O.P.D) while 48 (45%) were from In-door patient department (I.P.D). In the united states, UTIs account for seven million office visits and 1,00,000 hospitalisations annually, making them the most common bacterial infections in outpatient settings.[9]

The high prevalence of UTI may be due to factors like promiscuity, peer group of influence, pregnancy, low socio-economic status which are common among young men and women living in urban centres.[10] Sample obtained from female subjects (67%) yielded more bacteria than those obtained from males (33%). The sex distribution of the patients in the present study was consistent and well corroborated with earlier studies.[11,12] Several reports have indicated that females are more prone of having UTIs than males[10] because the urethra is shorter in females than males and more readily colonised by microorganisms. Women propensity to develop UTIs has also been explained on the basis of certain behavioural factors, including delays in micturition, sexual activity, the use of diaphragms and spermicides. Unlike females the length of the urethra, the dried environment surrounding the meatus, and the antibacterial properties of prostatic fluid contribute to a lower rate of infection in males. In present study more cases of UTI in females in age group 20-29 and 30- 39 depicted in table 3. This may be due to increased sexual activity among the reproductive age group of females predisposes them to the possibility of contracting UTI. Old age male patient having UTI more common than female in our study, due to some underlying clinical condition i.e, prostatitis, epididymis, orchitis, pyelonephritis, cystitis and other co-morbid conditions like long term use of catheter, urinary tract abnormalities and immunosuppressive treatments and others. So these results corroborate well with other earlier reports which showed that UTI are more common in females than males during adolescence.[13-16]

The uropathogens identified in our study are almost similar to those of many other studies conducted in different countries either in this region or internationally[17], however different results have also been reported by some earlier workers of different region. The Enterobacteriaceae family members were the most common microorganisms isolated from urinary tract infection in present study accounting 80% of the total isolated bacteria and *E. coli* (32.8 %) was found to be the most predominant bacteria. This results well corroborate with earlier studies.[2,18-22] However present study differs from some of the previous reports[23,29] who reported *P. aeruginosa* and *Klebsiella*s pp, respectively as the predominant bacteria. *P. aeruginosa* was also recorded as the second most common bacterial isolate in UTI from studies in India[13] and Nigeria.[15] However, *Klebsiella* spp was reported as the second most frequently implicated organism in UTI in some other studies.[2,14,23-25] The incidence of community acquired UTI due to *E. coli* is more in this study (68.5%) than hospital acquired infections (31.5%). *P. aeruginosa* was mostly responsible for UTI cases among hospitalised patients where it accounted for 7.4% of the infections. This is possibly due to the opportunistic nature and selection pressure of the organism and its versatility in causing nosocomial infections in hospitalized patients especially those who are with catheters. In present study *Staphylococcus aureus* is predominant bacteria among all gram positive cocci (10.2%) followed by Coagulase negative staphylococci (CoNS) (5.6%).

In our present study high prevalence of resistance with commonly used antibiotics such as ampicillin, amoxicillin, ciprofloxacin and ofloxacin has caused considerable alarm.[26,27] Most effective

antibiotics were found to be vancomycin, linezolid, nitrofurantoin gives 100% sensitive to all gram positive cocci whereas nalidixic acid and gentamicin gives 61% and 76% respectively. In case of gram negative bacilli imipenem, amikacin, netillin, piperacillin-tazobactam, tigecycline are found to be most effective drugs whereas ampicillin, amoxicillin-clavulanic acid are less effective. *E. coli*, the predominant etiologic organism of UTI in this study showed moderate to high susceptibility to the fluoroquinolones (ciprofloxacin and ofloxacin) and varying degree of susceptibility to other commonly used antibiotics. Earlier studies conducted in Nigeria[28], in Kuwait[29], in India[15] have reported good susceptibility of the bacteria to fluoroquinolones. However, resistance to fluoroquinolones is on the increase in the locality of our study.

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