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PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *ESCHERICHIA COLI* CAUSING URINARY TRACT INFECTION

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ABSTRACT

Urinary tract infection (UTI) is extremely common worldwide and numbers of patients are presenting to general practice and inpatient department. Gram negative bacteria are commonly involved in causing UTI. *Escherichia coli* is found to be the most common causative agent of UTI. Prudent and rational use of antimicrobial is possible by forming local, national and global wide antibiogram. This study is done to find out the prevalence and antimicrobial susceptibility pattern of *E.coli* causing urinary tract infection in tertiary care hospital, Bhopal. Total 1450 urine samples were collected and tested bacteriologically using standard procedures. Antimicrobial susceptibility testing was done by disk diffusion method described by Kirby-Bauer (1961). Culture positivity of urine samples was found to be 29 %.The most common pathogens were *E.coli* (62%) followed by *Klebsiella (15.4 %), Enterobacter (6.9%), Pseudomonas (5.7%), Staphylococci aureus (5.7%)* and others *(4.3%)*. *E.coli* was found to be most sensitive to imipenem followed by polymyxin-B, nitrofurantoin, gatifloxacin, colistin, doxycycline, amikacin and Gentamycin.

KEY WORDS: UTI, *E.coli*, antibiotic susceptibility testing, antimicrobial resistance.

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INTRODUCTION

Antimicrobial resistance has become a serious public health problem worldwide. Infections caused by resistant bacteria are associated with increased morbidity and mortality than those caused by susceptible pathogens[1, 2].Infections caused by resistant bacteria led to prolonged hospital stays, increased health care costs and in many cases to untreatable infections[3]. Infections of the urinary tract are extremely common worldwide and the numbers of patients are presenting to general practice and inpatient department[4].Urinary tract infections (UTIs) are the most common extra intestinal infections affecting people of all age groups and either sex[5]. Each year about 150 million people are diagnosed with UTI in all over the world[6]. Urinary tract infection is defined as the microbial invasion of any of the tissues of the urinary tract extending from the renal cortex to the urethral meatus. The urinary tract includes the organs that collect and store urine and release it from the body and these organs include the kidneys, ureters, bladder, urethra and accessory structures. *Escherichia coli* remained the most common causative agent of uncomplicated UTI for many years with 75-90% causes of UTI infection[7-9]. The other gram negative pathogens causing UTI are *Klebsiella spp., Proteus mirabilis and Pseudomonas aeruginosa, however, Enterococci* and coagulase negative *Staphylococci* are the most frequently encountered gram positive bacteria in UTI[10]. The antibiotic susceptibility patterns of UTI causing pathogens have been varying from time to time and from place to place in both community and hospital settings[11-13].Increasing drug resistance in pathogens is now a serious problem to treat diseases like malaria, tuberculosis, diarrheal diseases, and UTI etc[14].The main cause of this serious problem is the improper and uncontrolled use of antibiotics[15]as well as improper prescription, inappropriate dosage and duration of treatment[16].To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic susceptibility testing is mandatory[17]. Due to rising antibiotic resistance among uropathogens, it is important to have local hospital based knowledge of the organisms causing UTI and their antibiotic sensitivity patterns[18]. The present study was undertaken to find out the prevalence of E. coli infections in UTI and to determine the antimicrobial sensitivity pattern of *E.coli* infections among the patients attending People's Hospital of People's College of Medical Science & Research Centre,Peoples University, Bhanpur, Bhopal, M.P., India.

MATERIALS AND METHODS

In the present study 1450 urine samples were collected for the antibiotic sensitivity testing in the Department of Microbiology from inpatient & outpatient department of Peoples College of Medical Science & Research Centre and hospital, Bhopal from the period January 2010 to December 2011. First step done was to isolate the organisms from these urine samples and then to study the culture susceptibility in *Escherichia coli*. Identification of bacteria was done by gram staining. The samples were inoculated on MacConkey agar and Cystine Lactose Electrolyte Deficient (CLED) agar medium plates by four flame method. Inoculated culture plates were kept in the incubator for incubation at 37° C for 24 hours[19]. All the bacteria were identified using morphological, microscopy and biochemical tests following standard procedures described by Cowan and Steel (1974) and Cheesborough (2006)[20-21]. Antibiotic sensitivity testing (AST) was done only for pathogenic bacteria. Antibiotic sensitivity was performed by Kirby Bauer Disc Diffusion method [22].A sterile cotton swab was used to streak the surface of Mueller Hinton agar plates. Filter paper disks containing designated amounts of the antimicrobial drugs obtained from commercial supply firms (Himedia Labs, Mumbai, India) were used. The diameters of the zones of inhibition were measured by the unaided eye, including the diameter of the disc. AST of *E.coli* to different antibiotics is obtained. From AST antibiogram for *E.coli* is prepared.

RESULTS

During the 24 months period (January 2010 to December 2011), a total of 1450 urine samples were processed for culture and sensitivity testing. Urine samples of patients of all age groups (1day-85years) and both sexes were processed. A total 421different isolates were isolated from 1450 urine samples thus culture positivity was 29 % (421/1440) as shown in Table-1.

Table 1
Number and % of organisms, isolated from urine

Serial no	*Name of isolates*	Total no isolate out of (n = 421)	% of total isolates
1	*Escherichia coli*	262	62.0 %
2	*Klebsiella*	65	15.4 %
3	*Enterococci*	28	6.9 %
4	*Pseudomonas*	24	5.7 %
5	*Staphylococci aureus*	24	5.7 %
6	*Acinobactor*	07	1.7 %
7	*Citrobactor*	06	1.4 %
8	*Proteus sp.*	05	1.2 %
	Total	**421**	**100** %

Result in table-1 shows that out of total 421different organisms, *E.coli* was highest in number which accounted for 262(62%). Second highest isolates was *Klebsiella* which accounted 65 (14.5%) followed by *Enterococci* (6.9%)*, Pseudomonas* (5.7%), *Staphylococci aureus* (5.7 %), *Acinobactor* (1.7%), *Citrobactor* (1.4 %) and *Proteus sp.*(1.2 %).

Table 2
Age and sex wise distribution among total E.coli isolated

Serial no	Age group In years	Total age distribution of *E.coli* in Male	% distribution of *E.coli* in Male	Total age distribution of *E.coli* in Female	% distribution of *E.coli* in Female
1	0-10	20	21.3 %	10	5.9 %
2	11-20	18	19.1 %	13	7.7 %
3	21-30	22	**23.5 %**	55	**32.4 %**
4	31-40	2	2.1 %	30	17.8 %
5	41-50	6	6.3 %	30	17.8 %
6	51-60	6	6.3 %	21	12.5 %
7	>60	20	21.3 %	10	5.9 %
		94	100 %	**168**	100 %

Result in table-2 shows that out of 262 *E.coli* isolates, 168 (64.1%) were isolated from female and 94 (35.9%) were isolated from male; it showed the prevalence of *E.coli* infection is more in female than male. In this study it is found that maximum isolates were isolated from age group of 21-30 years in male and female both 32.4 % and 23.5 % respectively.

Figure 1
Antibiotic sensitivity of E.coli, isolated from urine

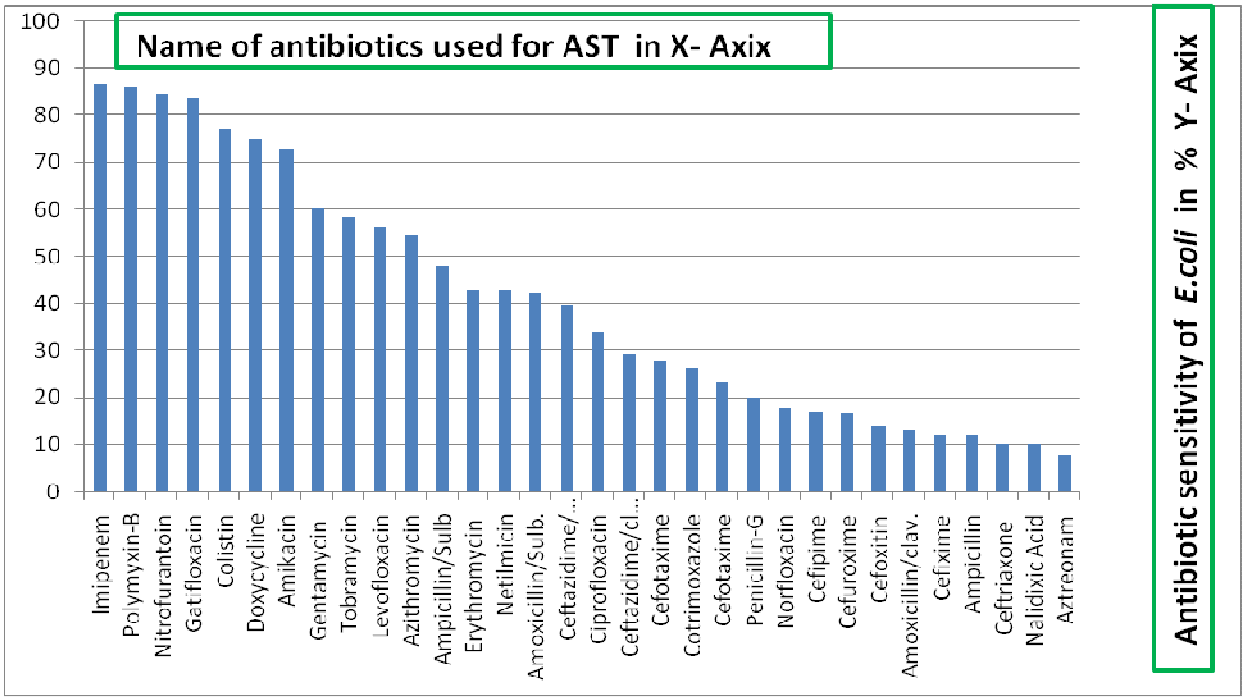

Table 3
Antibiotic Sensitivity of E.coli isolated from urine

Antibiotics used for AST	% Susceptibility	Antibiotic used for AST	% Susceptibility
Imipenem	86.8	Ciprofloxacin	34.0
Polymyxin-B	86.1	Ceftazidime/ Clav. Acid	29.3
Nitrofurantoin	84.5	Cefotaxime	28.0
Gatifloxacin	83.8	Cotrimoxazole	26.2
Colistin	77.0	CTX- C	23.3
Doxycycline	75.0	Penicillin-G	20.0
Ceftazidime	72.8	Norfloxacin	17.8
Amikacin	72.7	Cefipime	17.0
Gentamycin	60.0	Cefuroxime	16.7
Tobramycin	58.3	Cefoxitin	14.0
Levofloxacin	56.0	Amoxicillin/Clav. Acid	13.3
Azithromycin	54.5	Cefixime	12.0
Ampicillin/Sulbactam	48.0	Ampicillin	12.0
Erythromycin	43.0	Ceftriaxone	10.0
Netilmicin	42.9	Nalidixic Acid	10.0
Amoxicillin/Sulb.	42.2	Aztreonam	07.7

Result in table-3 and figure-1 show that *E.coli* is most sensitive to imipenem (86.8%) followed polymyxin-B (86.1%), nitrofurantoin (84.5%), gatifloxacin (83.8%), colistin (77 %), doxycycline (75 %), ceftazidime (72.8%), amikacin (72.7%), Gentamycin (60 %), tobramycin (58.3%), levofloxacin (56 %), azithromycin (54.5 %), ampicillin/ sulbactam (48 %).*E.coli* was found to be less sensitivity (resistance) to cefixime (12 %), ampicillin (12 %), ceftriaxone (10 %), nalidixic Acid (10 %) and aztreonam (7.7 %) as shown in table-3.

Table 4
Comparison of antibiotic sensitivity of E.coli isolated from urine with previous study

Serial Number	Name of antibiotics Used to treat *E.coli.*	% sensitivity of antibiotic in previousstudy[23]	% sensitivity of antibiotic in present study
1	Amikacin	90.6	72.7
2	Ampicillin	34.6	12
3	Cefipime	---	17
6	Cefotaxime	61.3	28
7	Cotrimoxazole	38.6	26.2
8	Gatifloxacin	---	83.8
9	Gentamicin	77.3	60
10	Imipenem	100	86.8
11	Nalidixic acid	42.6	10.0
12	Nitrofurantoin	77.3	84.5
13	Norfloxacin	56	17.8

Comparison of antibiotic sensitivity of *E.coli* isolated from urine of presentstudy with study done by Girishbabu et al[23] show that *E.coli* is havinggood sensitivity to imipenem, nitrofurantoin, gentamicin and amikacin in both studies. In present study, *E.coli* is less sensitive to norfloxacin, nalidixic acid, ampicillin, cefotaxime and cotrimoxazolein comparison to antibiotic sensitivity in above study.

Table 5
Comparison of antibiotic sensitivity of E.coli isolated from urine with previous study

Serial Number	Name of antibiotics Used to treat *E.coli.*	% sensitivity of antibiotic in previous study[24].	% sensitivity of antibiotic in present study
1	Imipenem	92.7	86.8
2	Amikacin	100	72.7
3	Cefotaxime	53.7	28
4	Ceftriaxone	51.2	10
5	Ciprofloxacin	34.1	34
6	Cotrimoxazole	34.1	26.2
7	Gatifloxacin	48.8	83.8
8	Gentamycin	78	60
9	Levofloxacin	63.4	56
10	Nalidixic Acid	4.8	10
11	Netilmicin	82.9	42.9
12	Nitrofurantoin	65.9	84.5
13	Tobramycin	82.9	58.3

Comparison of antibiotic sensitivity of *E.coli* isolated from urine of presentstudy with study done by Devanand Prakash et al[24] show similarities in antibiotic sensitivity exept *E.coli* is more sensitive to nitrofurantoin and gatifloxacin and less sensitive to amikacin, tobramycin and netilmicin in present study.

Table 6
Comparison of antibiotic sensitivity of E.coli isolated from urine with previous study

Serial Number	Name of antibiotics	% sensitivity of antibiotic previous study[25]	% sensitivity of antibiotic in present study
1	Amikacin	43.8	72.7
2	Ampicillin	6.8	12
3	Cefipime	54.7	17
6	Cefotaxime	48.5	28
7	Cotrimoxazole	12	26.2
8	Gatifloxacin	16.7	83.8
9	Gentamicin	33.3	60
10	Imipenem	92.7	86.8
11	Nalidixic acid	6.8	10.0
12	Nitrofurantoin	23.5	84.5
13	Norfloxacin	14.1	17.8

Comparison of antibiotic sensitivity of *E.coli* isolated from urine of presentstudy with study done by Swati Banerjee study[25] show *E.coli* is more sensitive to amikacin, nitrofurantoin, nalidixic acid, norfloxacin, ampicillinand gentamicin in present study. In both studies, *E.coli* is most sensitive to imipenem.

DISCUSSION

Bacterial urinary tract infection is one of the serious issues which need an urgent medical attention in the community. In the present study, the isolation rate of bacteria from urine was 29 % which is different from other reports[25].The most predictable and primary etiological bacteria involved in UTI is *Escherichia coli* in both out and inpatients[26-29].In this study, *E.coli* was the most common bacteria isolated from urine samples and this finding is in agreement with others finding too[30-37]. The prevalence of UTI occurred more in females than in malessecondary to shorter urethra, closer proximity to perirectal area in females. Out of the 262, *E.coli* isolates obtained, 168 were from females while 94 were from males. These results also agree with other reports, which showed that UTIs are more frequent in females than males during adulthood[38-40]. Urinary tract infections are one of the most commonly diagnosed infections in our hospital and probably in every hospital set up.In males, major age group was 21-30 years with positive urine culture in *E.coli* followed by more than 60 years of age. In older men, the incidence of UTI may increase due to prostatic obstruction or subsequent instrumentation like folly's catheter[41]. In females, major age group was 21-30 years with positive urine cultures in *E.coli*. It seems in females the incidence of UTI is seen was more at earlier age compared to that in males. UTIs are caused by a variety of microorganisms, including both gram positive and gram negative ones. The etiology of UTI has been regarded as well established & reasonably consistent. In our study *E.coli* (62 %) was predominant isolate followed by *Klebsiella spp.* (15.4%)*, Enterococci* (6.9%)*, Ps. Aeruginosa* (5.7%)*, staphylococci* (5.7%) *and Proteus spp.* (1.2 %)*.* This finding agrees with other reports which indicated that gram negative bacteria mostly *E. coli*&*Klebsiella Spp.* are the common pathogens isolated in patients with urinary tract infections[42-49].The most useful antibiotics having least resistance in this study were Imipenem, polymyxin-B, nitrofurantoin, gatifloxacin, colistin, doxycycline, ceftazidime, amikacin, gentamycin, tobramycin, levofloxacin and azithromycin. Least sensitive antibiotic having more resistance were cefixime, ampicillin, ceftriaxone, nalidixic acid and aztreonam. These antibiotics were used inappropriately had shown resistance. Similar findings were observed by many workers around the world[42-49].The possible explanation behind the resistance is showed to these antibiotics, may be because these antibiotics have been in use for a long period and must have been abused and as a result the organisms must have developed a different mode of action.

The study showed that the co-trimoxazole, nalidixic acid and norfloxacin are the drugs most commonly used for inpatients, which is reflected by the noticeable resistance shown by *E.coli* isolates to these antibiotics. The most useful antibiotics in this study were Imipenem 86.8%. This drug is relatively expensive when compared to most antibiotics frequently used. This probably had restricted their procurement and indiscriminate use, therefore making the organisms susceptible to it. Alarming finding seen in this study was that resistance shown to various third generation cephalosporins. Overall resistance to various generations of cephalosporins was high on account of the production of extended spectrum β-lactamases (ESBLs) by the *E.coli*. The resistance may also be due to the production of metallo-β-lactamases (MBL), which can be chromosomally encoded or plasmid mediated. The dose as well as the incidence of toxicity subsequently reduced if beta lactamase inhibitors are used with β-lactam antibiotics. The findings in present the study suggest that there is an urgent need for constant monitoring of susceptibility of pathogens in different populations to commonly used anti-microbial agents. The data of this study may be used to determine trends in antimicrobial susceptibilities, to formulate local antibiotic policies and overall to assist clinicians in the rational choice of antibiotic therapy to prevent misuse, or overuse, of antibiotics.

CONCLUSION

The study has showed that antimicrobial susceptibility testing reports is required before start of antibiotic treatment in cases of suspected UTI. The knowledge of antimicrobial pattern of routinely isolated uropathogens in that particular area may provide guidance to clinicians regarding the empirical treatment of UTI when therapy must be started before laboratory reports are available. *E.coli* was found to be most sensitive to imipenem followed polymyxin-B, nitrofurantoin, gatifloxacin, colistin, doxycycline, amikacin, Gentamycin, tobramycin, levofloxacin and azithromycin. *E.coli* was found to be least sensitive to cefixime, ampicillin, ceftriaxone, nalidixic Acid and aztreonam. Amikacin, colistin, doxycycline, levofloxacin and nitrofurantoin are found to be alternatives at low cost empirical therapy for treatment of UTI caused by *E.coli*. *E.coli* is highly sensitive for Imipenem but it is costlier so it should be reserved for multidrug resistant UTI. Strict adherence to the hospital antibiotic policy and good infection control practices can play a significant role in reducing the emerging drug resistance.Gatifloxacin remains the drug of choice for empiric treatment of UTI. Amikacin and nitrofurantoin are found to be alternatives at low cost. We conclude that laboratories should encourage accurate bacteriological record keeping of UTI isolates and their antibiogram for better management of these cases.

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