ORIGINAL ARTICLE

THE COMMONEST UROPATHOGENS CAUSING URINARY TRACT INFECTION AMONG PATIENTS ATTENDING A TERTIARY CARE HOSPITAL IN EASTERN BIHAR, AND THEIR ANTIBIOGRAM

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ABSTRACT: BACKGROUND: Urinary tract infections (UTI) are one of the most common infections in humans. Antimicrobial drug resistance is one of the major threats due to wide spread use of inappropriate and empirical antibiotic therapy. The present study highlights the organisms causing UTI and their antimicrobial susceptibility pattern among patients attending a Tertiary Care Hospital in Eastern Bihar. **MATERIALS AND METHODS:** A total of three hundred and ninety two (392) samples of urine from patients attending different inpatient and outpatient departments were included in the study. Urine samples were inoculated on Nutrient agar, Blood agar and McConkey agar plates by streaking. Inoculated plates were then incubated aerobically at 37^0C for 24 hours. After 24 hours of incubation, isolated colonies were picked up and Gram staining was done. Motility test and other biochemical tests were done for further identification of bacterial isolates using suitable Controls. Finally Antibiotic Susceptibility Test (AST) was performed to detect the degree of sensitivity or resistance of the pathogen isolated from the patient to an appropriate range of antimicrobial drugs on Mueller-Hinton agar (MHA) plates by Kirby-Bauer disc diffusion technique. **RESULTS AND CONCLUSION:** Out of the total of three hundred and ninety two (392) samples from an equal number of patients received and examined in the laboratory during the study period only one hundred and thirty seven (137) patients were found to have bacteriological infection. Among the bacteriologically positive cases, UTI was more common in females. Among the bacterial isolates *Escherichia coli* was the commonest pathogen in both males and females, followed by *Staphylococcus saprophyticus* in males, and *Staphylococcus aureus* in females. Gatifloxacin was the most effective antibiotic *in vitro* for the Gram Negative bacilli isolated, while Azithromycin was most effective against the Gram Positive cocci. The Gram Negative uropathogens showed a high degree of resistance to cephalosporins, while the Gram Positive cocci showed highest resistance to Norfloxacin, and also to a lesser extent, to the cephalosporins. It is due to the excessive use of antimicrobials for all sorts of infections, that uropathogens responsible for UTI are increasingly showing resistance to antibiotics. The knowledge of uropathogens and their antimicrobial susceptibility pattern in this geographical region will help in appropriate and judicious antibiotic usage in our health care setup.

KEYWORDS: Uropathogen, Urinary Tract Infection, Bacterial resistance, Cephalosporins, Fluoroquinolones, Antibiotic Susceptibility Pattern.

INTRODUCTION: Acute urinary tract infection (UTI) is a common condition encountered in day to day medical practice. It affects patients of all age groups, in both sexes and varies in severity from an unsuspected infection to a condition of severe systemic disease. UTI, which is defined as presence and active multiplication of microorganisms within the urinary tract, is one of the commonest

bacterial infections seeking treatment in clinical practice. Although a variety of aetiology is involved with UTI, *Escherichia coli* and other coliforms account for a large majority of naturally acquired urinary tract infections. They are also a frequent cause of nosocomial infection in many hospitals [1, 2].

A patient is said to have a urinary tract infection, when there is the presence of over $1x10^5$ organisms per ml in the midstream sample of urine [3]. The human urinary tract is a collecting and emptying system, which comprises of the kidneys, the ureters, bladder and urethra. Infections in any of these anatomical sites are referred to as UTI. Infections extending to the bladder leads to cystitis while those involving the kidneys leads to pyelonephritis [4]. *Escherichia coli* is the most common cause of urinary tract infection [5, 6] and accounts for approximately 90% of first urinary tract infection in young women [6]. The symptoms and signs include increased urinary frequency, dysuria, hematuria and pyuria. Flank pain is associated with upper tract infections. None of these symptoms or signs is specific for *Escherichia coli* infection [7]. Urinary tract infection can result in bacteriuria with clinical signs of sepsis [8].

Experimentally, a hundred thousand bacterial counts per millilitre of urine is indicative of a urinary tract infection, though lesser counts may be strongly suggestive in some instances, especially among pregnant women, where asymptomatic UTI could predispose them to greater risk of developing symptomatic UTI and its associated obstetric complications [9].

Bacteriological investigations of UTI are not complete without an antibiotic sensitivity test of the isolate. Microorganisms causing UTI vary in their susceptibility to antimicrobials from place to place and time to time. The present study was undertaken to study the prevalence of urinary tract infections among the patients in a tertiary care hospital in eastern Bihar. This is the first report of such kind from this institute.

MATERIAL AND METHODS: The present study was a pilot study conducted in our department over a period of 3 months from April to June 2013. Subjects for the study were randomly selected and included patients suffering from UTI, from all age groups. There was no bias in selection of cases as far as gender, socio-economic or religious backgrounds were concerned.

A total of three hundred and ninety two (392) samples of urine from patients attending different inpatient and outpatient departments were included in the study. A brief clinical history of the patients and antibiotic intake, if any, was taken. Specimen collected were mid-stream urine samples from the suspected cases of UTI. Urine was collected in sterile plastic containers, with all aseptic precautions. The specimens collected were examined by microscopy and then put up for culture. The samples were inoculated on Nutrient agar, Blood agar and McConkey agar plates by streaking. Inoculated plates were then incubated aerobically at 37^0C for 24 hours. After 24 hours of incubation, isolated colonies were picked up and Gram staining was done. Motility test and other biochemical tests were done for further identification of bacterial isolates. Control strains used were as follows:

Escherichia coli: ATCC 25922
Klebsiella pneumonia: ATCC 700603
Proteus mirabilis: ATCC 7002
Pseudomonas aeruginosa: ATCC 27853
Staphylococcus aureus: ATCC 25923
Staphylococcus saprophyticus: ATCC 15305
Staphylococcus epidermidis: ATCC 14990
Enterococcus faecalis: ATCC 29212

Finally, Antibiotic Susceptibility Test (AST) was performed to detect the degree of sensitivity or resistance of the pathogen isolated from the patient to an appropriate range of antimicrobial drugs. AST was done on Mueller-Hinton agar (MHA) plates by Kirby-Bauer disc diffusion technique [10] using commercially available antibiotic discs (HiMedia, Mumbai). Interpretation of results was done based on the diameter of the zone of inhibition as per guidelines laid down by CLSI (Clinical and Laboratory Standards Institutes). The antibiotics and the concentrations at which they were used were as follows:

Sparfloxacin (5 µg)	Norfloxacin (10 µg)	Cefuroxime (30 µg)
Cephalexin (30 µg)	Ofloxacin (5 µg)	Gentamycin (10 µg)
Azithromycin (15 µg)	Cotrimoxazole (25 µg)	Gatifloxacin (5 µg)
Cirofloxacin (5 µg)	Amoxycillin (30 µg)	Ceftriaxone (30 µg)
Amikacin (30 µg)		

RESULTS: Out of the total of three hundred and ninety two (392) samples from an equal number of patients received and examined in the laboratory during the study period only one hundred and thirty seven (137) patients were found to have bacteriological infection (34.95%). The remaining samples of two hundred and fifty five (255) patients were considered sterile (65.05%) **[Table 1]**.

Out of the one hundred and thirty seven (137) positive samples, 51 (37.23%) were male patients and 86 (62.77%) were female patients **[Table 2]**. From this table it is clear that the bacteriological evidence of urinary tract infection was more in females.

Table 3 shows that amongst the one hundred and thirty seven (137) bacteriologically infected samples, *Escherichia coli* was the commonest pathogen (52.55%) responsible for urinary tract infection followed by *Staphylococcus aureus* (18.25%), *Staphylococcus saprophyticus* (8.76%), *Staphylococcus epidermidis* (5.84%), *Klebsiella pneumoniae* (3.65%), *Enterococcus faecalis* (3.65%), *Proteus mirabilis* (2.92%) and *Pseudomonas aeruginosa* (2.92%).

As evidenced from the same Table, It is also clear that the main cause of UTI in both males (50.98%) and females (53.49%) was *Escherichia coli* followed by *Staphylococcus saprophyticus* (13.72%), *Staphylococcus aureus* (9.80%), *Staphylococcus epidermidis* (7.84%), *Pseudomonas aeruginosa* (5.88%) *Klebsiella pneumoniae* (3.92%), *Enterococcus faecalis* (3.92%) and *Proteus mirabilis* (1.96%) in males, and, *Staphylococcus aureus* (23.26%), *Staphylococcus saprophyticus* (5.81%), *Staphylococcus epidermidis* (4.65%), *Klebsiella pneumoniae* (3.49%), *Enterococcus faecalis* (3.49%), *Proteus mirabilis* (3.49%) and *Pseudomonas aeruginosa* (1.16%) in females. The most important uropathogens responsible for UTI, therefore, were *Escherichia coli*, *Staphylococcus saprophyticus*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Proteus mirabilis* were implicated only in a small percentage of cases among the samples studied. A mixed involvement of *Escherichia* coli and *Staphylococcus aureus* was seen in only a negligible number of cases.

Out of seventy two (72) strains of *Escherichia coli* isolated from the urine culture, 56 strains were found to be sensitive to Gatifloxacin (77.78%), followed by Sparfloxacin (76.39%), Amikacin (62.5%), Cotrimoxazole (54.17%), Gentamycin (51.39%), Ceftriaxone and Ciprofloxacin (45.83% each) and Ofloxacin (40.28%). The remaining antibiotics were effective in less than 25% strains **[Table 4]**.

Out of twenty five (25) strains of *Staphylococcus aureus* isolated from the urine culture, 24 were found to be sensitive to Azithromycin (96%), followed by Sparfloxacin (80%), Ciprofloxacin (68%), Ceftriaxone (64%), Gatifloxacin (60%), Cotrimoxazole (52%), Cefuroxime (44%), Amikacin and Gentamycin (40% each), Ofloxacin (36%) and Cephalexin (28%). The remaining antibiotics were effective in less than 25% strains **[Table 4]**.

Out of twelve (12) strains of *Staphylococcus saprophyticus* isolated from the urine culture, 12 were found to be sensitive to Azithromycin (100%), followed by Sparfloxacin (83.33%), Amikacin (75%), Ciprofloxacin and Ofloxacin (66.67% each), Cefuroxime (58.33%), Gatifloxacin (50 %), Gentamycin (41.67%), Ceftriaxone (33.33%), Amoxycilline and Norfloxacin (25% each). Cephalexin and Cotrimoxazole were effective in less than 25% strains **[Table 4]**.

High degree of resistance was seen in *E. coli* with reference to Azithromycin (85%), Cephalexin (72%), Norfloxacin (69%), Amoxyciline (67%), Cefuroxime (61%), Ofloxacin (60%), Ceftriaxone and Ciprofloxacin (54% each). Ciprofloxacin resistance was more in *E. coli* as compared to resistance seen in the other Gram Negative uropathogens **[Table 4]**.

The rates of resistance among the Gram Negative uropathogens isolated to cephalosporins like Cephalexin, Cefuroxime and Ceftriaxone was high. Ceftriaxone resistance was 54% in *E. coli*, 60% in among Klebsiella, 75% in Proteus and 50% in Pseudomonas **[Table 4]**.

Amongst the Gram Positive isolates the commonest uropathogen was *Staphylococcus aureus* (18.25%), while the rarest isolate was *Enterococcus faecalis* (3.65%). While most isolates of *Staphylococcus aureus* were resistant to Norfloxacin (92%), *Enterococcus faecalis* was most resistant to the Cephalosporins like Cephalexin, Cefuroxime and Ceftriaxone **[Table 4]**.

DISCUSSION AND CONCLUSION: This study showed that *E. coli* was the commonest pathogen causing complicated and uncomplicated UTI as described previously [11-13] amongst the several organisms known to cause UTI, including *P. aeruginosa, S. saprophyticus, S. epidermidis,* Enterococcus spp.*, P. mirabilis, Klebsiella pneumonia etc.* as reported by earlier workers [14, 15]. This study also demonstrates (Table 3) the involvement of *E. faecalis* in causing UTI. Among the non-fermenters *Pseudomonas aeruginosa* was isolated as an uropathogen particularly in the intensive care units, although in a very few cases (2.92%). Acinetobacter and Citrobacter, both common pathogens in UTI, were however not isolated in this present study. Such findings have been documented elsewhere [16-27, 28-31]. Furthermore, while most other workers elsewhere have reported the involvement of Klebsiella as the second most important pathogen in UTI cases [16-27, 28-31], our present study in this area shows a far greater involvement of gram-positive cocci than Klebsiella.

While ciprofloxacin and ofloxacin are the most extensively used fluoroquinolones for the treatment of UTIs the emergence of resistance for fluoroquinolones is based on several factors [15, 19, 29, 30]. Resistance to ciprofloxacin has emerged in a variety of genera belonging to the family Enterobacteriaceae. Our findings concur with such findings reported earlier [32, 33]. Apart from the notable resistance of *E. coli* to ciprofloxacin, other organisms were also found to be resistant to ciprofloxacin especially *K. pneumoniae*, Pseudomonas spp., Proteus spp., Enterobacter spp., Staphylococcus spp, and *E. faecalis*. Also, fluoroquinolone resistance in *E. coli* has emerged particularly in patients with urinary tract infections who have received fluoroquinolone prophylaxis [17-24]. An association between the increase in quinolone prescriptions and an increase in bacterial resistance has been reported from several countries [17-20, 24]. Usually, the prevalence of

fluoroquinolone resistance is related to the intensity of antibiotic use [17]. Resistance rates for ciprofloxacin against uncomplicated UTI pathogens were reported as 0-14.7% in the ECO-SENS Project, 2.5% in the USA and 1.2% in outpatients in Canada [12, 18, 19].

In conclusion, the present results in increasing antibiotic resistance trends amongst UTI patients indicate that it is imperative to rationalize the use of antimicrobials and to use these conservatively. Due to excessive use of antimicrobials for all sorts of infections, uropathogens responsible for UTI are increasingly showing resistance to antibiotics. The knowledge of uropathogens and their antimicrobial susceptibility pattern in this geographical region will help in appropriate and judicious antibiotic usage in our health care setup.

Table 1: Distribution of sterile samples & sample showing bacteriological evidence of UTI

Samples	Total number of samples under study (n = 392)	
	Number	Percentage (%)
Sterile samples	255	65.05
Samples (isolates) showing bacteriological evidence of UTI	137	34.95

Table 2: Gender-wise distribution among the cases suffering from UTI

Sex	Total number of isolates under study (n = 137)	
	Number	Percentage (%)
Male	51	37.23
Female	86	62.77

Table 3: Gender-wise distribution of different pathogenic organism among cases of UTI

Pathogenic Organisms	Total isolates under study (n = 137)		Total isolates in males (n=51)		Total isolates in Females (n=86)	
	Number	Percentage	Number	Percentage	Number	Percentage
Escherichia coli	72	52.55	26	50.98	46	53.49
Klebsiella pneumoniae	5	3.65	2	3.92	3	3.49
Proteus mirabilis	4	2.92	1	1.96	3	3.49
Pseudomonas aeruginosa	4	2.92	3	5.88	1	1.16
Staphylococcus aureus	25	18.25	5	9.80	20	23.26
Staphylococcus saprophyticus	12	8.76	7	13.72	5	5.81
Staphylococcus epidermidis	8	5.84	4	7.84	4	4.65
Enterococcus faecalis	5	3.65	2	3.92	3	3.49
Mixed (*Escherichia coli & Staphylococcus aureus*)	2	1.46	1	1.96	1	1.16

Table 4: The sensitivity pattern of UTI isolates and their antibiogram

SL. No.	Antibiotic	Number of Sensitive strains							
		Gram Negative Bacilli				Gram Positive cocci			
		Escherichia coli (n=72)	*Klebsiella pneumonia* (n=5)	*Proteus mirabilis* (n=4)	*Pseudomonas aeruginosa* (n=4)	*Staphylococcus aureus* (n=25)	*Staphylococcus saprophyticus* (n=12)	*Staphylococcus epidermidis* (n=8)	*Enterococcus faecalis* (n=5)
1	Sparfloxacin	55 (76.3)	4 (80.00)	1 (25.00)	2 (50.00)	20 (80.00)	10 (83.33)	5 (62.50)	5 (100.00)
2	Cephalexin	15 (28.8)	1 (20.00)	0 (00.00)	1 (25.00)	7 (28.00)	2 (16.67)	3 (37.50)	1 (20.00)
3	Azithromycin	11 (15.28)	0 (00.00)	0 (00.00)	0 (00.00)	24(96.00)	12 (100.00)	6 (75.00)	4 (80.00)
4	Ciprofloxacin	33 (45.83)	3 (60.00)	2 (50.00)	2 (50.00)	17(68.00)	8 (66.67)	5 (62.00)	3 (60.00)
5	Amikacin	45 (62.50)	2 (40.00)	2 (50.00)	2 (50.00)	10(40.00)	9 (75.00)	1(12.50)	3 (60.00)
6	Norfloxacin	22 (30.56)	3 (60.00)	1 (25.00)	3 (60.00)	2 (8.00)	3 (25.00)	1(12.50)	2 (40.00)
7	Ofloxacin	29 (40.28)	3 (60.00)	3 (75.00)	1 (25.00)	9 (36.00)	8 (66.67)	2(25.00)	2 (40.00)
8	Cotrimoxazole	39 (54.17)	2 (40.00)	3 (75.00)	1 (25.00)	13 (52.00)	2 (16.67)	2(25.00)	2 (40.00)
9	Amoxicillin	24 (33.33)	3 (60.00)	2 (50.00)	1 (25.00)	6 (24.00)	3 (25.00)	3(37.50)	2 (40.00)
10	Cefuroxime	28 (38.89)	4 (80.00)	1 (25.00)	3 (60.00)	11(44.00)	7 (58.33)	4(50.00)	0 (00.00)
11	Gentamycin	37 (51.39)	2 (40.00)	2 (50.00)	3 (60.00)	10 (40.00)	5 (41.67)	4(50.00)	2 (40.00)
12	Gatifloxacin	56 (77.78)	3 (60.00)	4 (100.00)	2 (50.00)	15 (60.00)	6 (50.00)	5(62.50)	3 (60.00)
13	Ceftriaxone	33 (45.83)	2 (40.00)	1 (25.00)	2 (50.00)	16 (64.00)	4 (33.33)	4(50.00)	1 (20.00)

Figures in parenthesis indicate percentages of total isolates.

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