Emergence of High Level Multi-Drug Resistance in Bacterial Isolates from UTI Cases Attending a Tertiary Care Hospital in North India

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Abstract

introduction: Urinary tract infection (UTI) is one of the commonest causes of morbidity worldwide. Etiology and antibiotic resistance pattern of uropathogens keeps changing with time. This study was an attempt to estimate the prevalence and antimicrobial resistance pattern of various bacterial isolates in patients attending a tertiary care hospital in north India.

Materials & Methods: Total 307 urine samples were included in this study from January 2011 to December 2011 and 104 samples showed significant colony count of pathogens. Urinary isolates were identified by conventional methods and antibiotic susceptibility test of each isolate was performed by Kirby-Bauer disc-diffusion method according to CLSI guidelines.

Results: Gram-negative isolates (73.12%) pre-dominated compared to gram-positive ones (26.88%). The most prevalent bacteria were *Escherichia coli* (45.26%) followed by *Klebsiella* spp. (18.26%), *Staphylococcus aureus* (17.3%), *Enterococcus* spp., *Citrobacter* spp. The majority of the isolates were from females (71.15%) while the remaining was from males (28.84%). Among the gram-negative enteric bacilli, high prevalence of resistance was observed against first line antibiotics namely ampicillin, ampicillin-sulbactum, co-trimoxazole, norfloxacin, levofloxacin, nitrofurantoin, cefuroxime, ceftazidime, cefoperazone-sulbactum, gentamicin and amikacin. Carbapenems, especially impenem was most sensitive in 90% cases. Among 9 *Enterococcus* isolates, all were resistant to ampicillin while 4(44.45%) to ampicillin-sulbactum, 6 (66.67%) to levofloxacin and 7 (77.78%) to gentamicin. Among 18 *Staphylococcus aureus* isolates 16 (88.89%) were MRSA. No Vancomycin resistance was observed in either *Enterococcus* or *Staphylococcus* isolates.

Conclusion: Multi-drug resistance in common uropathogens is emerging at an alarming rate, making urine culture and sensitivity a mandatory test for appropriate management of a very common disease like UTI.

Keywords: antimicrobial resistance, bacterial isolates, UTI, North India

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common types of bacterial infections in humans occurring both in the community and health care settings. UTI ranks the highest among the most common reasons that compel an individual to seek medical attention[1]. Today it represents one of the most common diseases encountered in medical practice, affecting people of all ages, from the neonate to the geriatric age group[2].

The term urinary tract infection (UTI) denotes several distinct entities with the common feature of significant pyuria and bacteriuria[3]. The causative pathogen profile varies from region to region, but *Escherichia coli (E. coli)* remains the most common causative pathogen. The sensitivity of uropathogens to different drugs varies in different areas, and changes with time. This necessitates periodic studies of the causative uropathogens and their antibiotic sensitivity pattern[4].

UTIs are often treated with different broad-spectrum antibiotics when one with a narrow spectrum of activity may be appropriate because of concerns about infection with resistant organisms. Fluoroquinolone are preferred as initial agents for empiric therapy of UTI in area where resistance is likely to be of concern[4,5]. This is because they have high bacteriological and clinical cure rates, as well as low rates of resistance, among most common uropathogens[6-8]. The extensive uses of antimicrobial agents have invariably resulted in the development of antibiotic resistance, which, in recent years, has become a major problem worldwide[9]. Current knowledge on antimicrobial susceptibility pattern is essential for appropriate therapy. Emerging multidrug resistant strains is of major concern to treat UTI. This study was designed to evaluate the profile of isolates causing UTI and their resistance to various antimicrobial agents in patients attending the OPD and admitted in IPD, of a tertiary care hospital in North India.

MATERIALS AND METHODS

A total of 307 urine samples from outdoor and indoor patients attending our hospital from January 2011 to December 2011 were processed in the department of microbiology. The urine samples were inoculated on MacConkey agar or Blood agar by using the calibrated loop and were incubated aerobically for 18-24 hours at 37°C.Urinary isolates from symptomatic UTI cases were identified on the basis of colony morphology, Gram's staining, catalase test, oxidase test, coagulase test and standard biochemical tests. 104 (33.87%) samples had significant bacteriuria and were further processed for identification and antibiotic susceptibility test of the pathogen. Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method. The data was analyzed using Fischer's exact test and p value of < 0.05 was taken as statistically significant.

One specimen per patient was taken and patients with significant bacteriuria (≥10^5 cfu/ml) were included for the microbiological analysis. Unsterile, improperly stored or delayed specimen, were excluded from the study.

From the clinically suspected patients of UTI, midstream clean catch specimen of urine from both male and female patients was collected in a sterile, screw -capped, wide mouthed universal container. The bacterial count in the urine sample was determined by Semi-quantitative culture method using 3.26 mm internal diameter standard loop (Hi- Media laboratories Ltd.)[10].

Antimicrobial sensitivity testing of all significant isolates was performed by Kirby-Bauer disk diffusion method, according to CLSI 2011 guidelines[11]. Commercially available antibiotic disc of 6mm (Hi- Media lab) were used. Antibiotic disc were selected according to bacterial isolates. In the present study the antibiotic disc tested were: Ampicillin (10μg), Ampicillin/sulbactum (10/10μg), Co-trimoxazole (25 μg), Gentamicin (10μg), Nitrofurantoin (300 μg), Nalidixic acid (30μg), Norfloxacin (10μg), Ciprofloxacin (5μg), Amikacin (30μg), Levofloxacin (5μg), Cefotaxime (30μg), Cefotaxime/clavulanic acid (30/10μg), Ceftriaxone (30μg), Ceftriaxone/sulbactam (30/15μg), Ceftazidime (30μg), Ceftazidime/clavulanic acid (30/10μg), Imipenem/cilastin (10/10μg), Meropenem (10μg), Polymixin B and Tigecycline (15μg).

RESULTS

Total 307 samples were included in the present study to estimate the prevalence of Antimicrobial resistance in patients suffering from Urinary tract infection attending the OPD and IPD of the hospital. Total 104 (33.87%) samples showed significant colony count (10^5cfu/ml) of pathogens, 153 (49.8%) samples were sterile, 30 (9.8%) samples showed multiple isolates (samples grossly contaminated during collection) and 20 (6.5%) samples showed insignificant colony count. Out of 104 culture positive samples 58 (55.76%) were from IP and 46 (44.24%) were from OP patients.

All patients were between the ages of 1 to 85 years. Majority of patients were females 74 (71.16%) compared to males 30 (28.84%). Females of reproductive age group (between 21 and 40 years) constituted 52.89% of the total patients. However, males of >40 years, had a higher prevalence of 12.51% compared to females' 6.73% of the same age group. (Table 1 and Figure 1)

Out of total 104 isolates, majority were gram negative 77 (74.03%) while 27 (25.96%) were gram positive. *Escherichia coli* (*E. coli*) was the most common organism isolated from 47 (45.19%) cases, followed by *Klebsiella* spp. 19 (18.26%), *S. aureus* 18 (17.30%) and *Enterococcus* spp. 9 (8.65%). Uncommon bacterial isolates obtained in the study were *Citrobacter* spp., *Pseudomonas* spp.,

Table 1: Age and sex distribution of urinary tract infection cases

Age group (years)	n (%)			*P* value
	Total	Male	Female	
1-10	07 (6.73)	1 (0.96)	6 (5.77)	0.670
11-20	11 (10.57)	5 (4.80)	6 (5.77)	0.289
21-30	40 (38.46)	5 (4.80)	35 (33.66)	0.003*
31-40	27 (25.96)	7 (6.73)	20 (19.23)	0.807
41-50	12 (11.54)	6 (5.77)	6 (5.77)	0.099
51-60	04 (3.86)	3 (2.90)	1 (0.96)	0.071
61-70	01 (0.96)	1 (0.96)	0	N.A.
71-80	01 (0.96)	1 (0.96)	0	N.A.
81-90	01 (0.96)	1 (0.96)	0	N.A.
Total	104 (100)	30 (28.84)	74 (71.16)	0.001*

**P* value significant, < 0.05

Acinetobacter spp., *Proteus* spp., *Enterobacter* spp. and *CoNS* (Table 2 and Figure 2).

The antibiotic sensitivity pattern of gram negative isolates is shown in Table 3. In first line antibiotics, all gram negative isolates were resistant to ampicillin while cotrimoxazole was found to be sensitive only in 6 (12.8%) *E. coli* and 2 (10.5%)

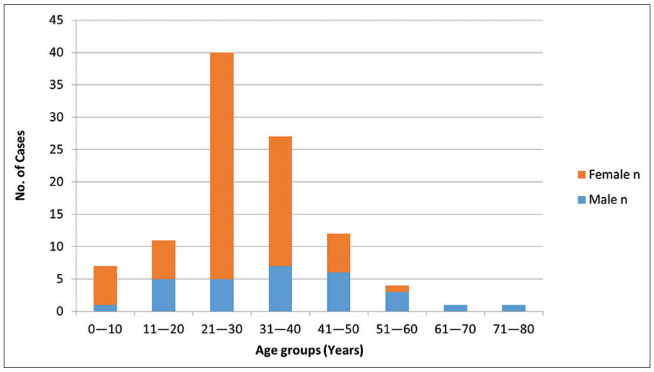

Figure 1: Bar diagram showing gender distribution of UTI cases

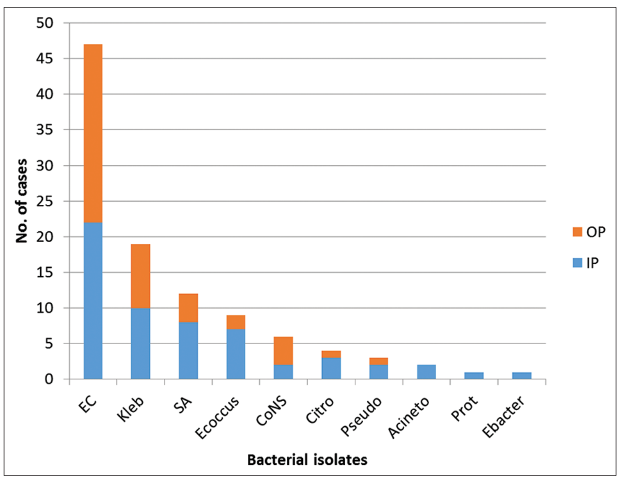

Figure 2: Distribution of various bacterial isolates in indoor and outdoor patients. Abbreviations: E.C.: *E. coli*, Kleb: *Klebsiella* spp., S.A.: *S. aureus*, Ecoccus: *Enterococcus* spp., CoNS: Coagulase Negative *Staphylococcus*, Citro: *Citrobacter* spp., Pseudo: *Pseudomonas* spp., Acineto: *Acinetobacter* spp., Prot: *Proteus* spp., Ebacter: *Enterobacter* spp

Klebsiella spp. isolates, nitrofurantoin in 10 (21.3%) *E. coli* and 4 (21%) *Klebsiella* spp. isolates, norfloxacin in 6 (12.8%) *E. coli*, 4 (21%) *Klebsiella* spp., 1 (25%) *Citrobacter* spp. and 1 (33.3%) *Pseudomonas* spp. isolates, gentamicin in 9 (9.1%) *E. coli*, 5 (26.3%) *Klebsiella* spp., one isolate each of *Citrobacter* spp. (25%), *Pseudomonas* spp. (33.3%) and *Acinetobacter* spp. (50%). In second line antibiotics, amikacin was found to be sensitive only in 7 (14.5%) *E. coli*, 2 (10.5%) *Klebsiella* spp., 1 (25%) *Citrobacter* spp. and 1 (100%) *Enterobacter* spp. isolate; ceftazidime in only 1(2.1%) *E. coli* isolate, cefuroxime in 1 (2.1%) *E. coli* and 2 (10.5%) *Klebsiella* spp. isolates, cefoperazone/sulbactum in 2 (4.2%) *E. coli*, 1 (5.25%) *Klebsiella* spp. and 1 (25%) *Citrobacter* spp. isolate. However, levofloxacin emerged as the most sensitive drug in second line group and was found to be sensitive in, 26 (55.1%) isolates of *E. coli* followed by 10 (52.6%) *Klebsiella* spp., but only 1 (25%) isolate of *Citrobacter* spp.

In reserved antibiotics category, majority of isolates of *E. coli* (72.3%) and rest of all other isolates were sensitive to imipenem, followed by meropenem in 29 (61.7%) *E. coli*, 13 (68.4%) *Klebsiella* spp., 3 (75%) *Citrobacter* spp., 2 (66.6%) *Pseudomonas* spp., 1 (50%) *Acinetobacter* spp., all isolates of *Enterobacter* spp. and *Proteus* spp. Most sensitive drugs were found to be polymixin B and tigecycline, with sensitivity in 44 (93.6%) and 42 (89.4%) isolates of *E. coli*, 16 (84.2%) and only 4 (21%) *Klebsiella* spp. isolates, 3 (75%) and 1 (25%) *Citrobacter* spp. isolates and all *Pseudomonas* spp., *Acinetobacter* spp., *Enterobacter spp.* and *Proteus* spp. isolates respectively (Table 3).

In gram positive isolates, only 2 (11.11%) *Staphylococcus* spp. were sensitive to ampicillin, followed by 4 (22.2%) to norfloxacin, 6 (33.3%) to cotrimoxazole and levofloxacin each, 7 (38.9%) to ampicillin/sulbactum, 10 (55.5%) to gentamicin, 14 (77.8%) to clindamycin. Only 2 (11.1%) *Staphylococcus* spp. isolates were cefoxitin sensitive. In *Enterococcus* spp. no isolate was sensitive to ampicillin, 2 (22.2%) to gentamicin, 3 (33.3%) to levofloxacin and 5 (55.5%) to ampicillin/sulbactum, All *Staphylococcus* spp.

Table 2: Frequency and distribution of bacterial isolates from UTI cases

Bacterial isolate	*n* (%)	IP	OP	*P* value
E. coli	47 (45.19)	22 (21.15)	25 (24.04)	0.114
Klebsiella spp.	19 (18.26)	10 (9.61)	9 (8.65)	0.802
S. aureus	12 (11.53)	8 (7.69)	4 (3.84)	0.544
Enterococcus spp.	9 (8.65)	7 (6.73)	2 (1.92)	0.292
Coagulase Negative Staphylococcus (CoNS)	6 (5.76)	2 (1.92)	4 (3.84)	0.401
Citrobacter spp.	4 (3.84)	3 (2.88)	1 (0.96)	0.627
Pseudomonas spp.	3 (2.88)	2 (1.92)	1 (0.96)	1.000
Acinetobacter spp.	2 (1.92)	2 (1.92)	0	N.A.
Proteus spp.	1 (0.96)	1 (0.96)	0	N.A.
Enterobacter spp.	1 (0.96)	1 (0.96)	0	N.A.
Total	104 (100)	58 (55.76)	46 (44.24)	0.127

Table 3: Antibiotic sensitivity pattern of gram negative isolates

Antibiotic	*E. coli* *n*=47 (%)	*Klebsiella* spp. *n*=19 (%)	*Citrobacter* spp. *n*=4 (%)	*Pseudomonas* spp. *n*=3 (%)	*Acinetobacter* spp. *n*=2 (%)	*Enterobacter* spp. *n*=1 (%)	*Proteus* spp. *n*=1 (%)
Ampicillin	0	0	0	0	0	0	0
Ampicillin/Sulbactum	5 (10.6)	2 (10.5)	0	0	0	0	0
Cotrimoxazole	6 (12.8)	2 (10.5)	0	0	0	0	1 (100)
Nitrofurantoin	10 (21.3)	4 (21)	0	0	0	0	0
Gentamicin	9 (19.1)	5 (26.3)	1 (25)	1 (33.3)	1 (50)	0	0
Norfloxacin	6 (12.8)	4 (21)	1 (25)	1 (33.3)	0	0	0
Ceftazidime	1 (2.1)	0	0	0	0	0	0
Cefuroxime	1 (2.1)	2 (10.5)	0	0	0	0	0
Amikacin	7 (14.9)	2 (10.5)	1 (25)	0	0	1 (100)	0
Levofloxacin	26 (55.1)	10 (52.6)	1 (25)	0	0	0	0
Cefoperazone/Sulbactum	2 (4.2)	1 (5.25)	1 (25)	0	0	0	0
Imipenem	34 (72.3)	19 (100)	4 (100)	3 (100)	1 (50)	1 (100)	1 (100)
Meropenem	29 (61.7)	13 (68.4)	3 (75)	2 (66.6)	1 (50)	1 (100)	1 (100)
Polymixin B	44 (93.6)	16 (84.2)	3 (75)	3 (100)	2 (100)	1 (100)	1 (100)
Tigecycline	42 (89.4)	4 (21)	1 (25)	3 (100)	2 (100)	1 (100)	1 (100)

and *Enterococcus* spp. isolates were sensitive to vancomycin (Table 4).

Table 4: Antibiotic sensitivity pattern in gram positive isolates

Antibiotic	*Staphylococcus* spp. N=18 (%)	*Enterococcus* spp. N=9 (%)
Ampicillin	2 (11.1)	0
Ampicillin/Sulbactum	7 (38.9)	5 (55.5)
Cotrimoxazole	6 (33.3)	-
Cefoxitin	2 (11.1)	-
Norfloxacin	4 (22.2)	-
Levofloxacin	6 (33.3)	3 (33.3)
Gentamicin	10 (55.5)	2 (22.2)
Clindamycin	14 (77.8)	-
Vancomycin	18 (100)	9 (100)

DISCUSSION

Urinary tract infections are one of the most common types of bacterial infections occurring in humans[12]. This study was undertaken to identify pathogenic bacteria responsible for urinary tract infection and to determine their antimicrobial resistance pattern.

Out of the total 307 urine samples, 104 (33.9%) samples yielded significant colony count, 153 (49.8%) samples were sterile, 30 (9.8%) samples showed multiple isolates (samples grossly contaminated during collection) and 20 (6.5%) samples showed insignificant colony count. Our results are similar to a previous study done by Mandal *et al.*[13], who reported 62% samples sterile, 26.01% showed significant growth, 2.3% showed insignificant growth and 9.6% were found contaminated. It shows that culture positivity rate varies from one laboratory to other.

Higher prevalence of UTI in females than in males, is a well recognized fact; and our findings in accordance with culture positivity in 74 (71.2%) females compared to only 30 (28.8%) in males. However, a reversal of prevalence was seen in >50 years age groups, where higher prevalence of 5.8% was found in males compared to only 0.96% in females. This might be due to decrease in prostatic secretions in males and decreased sexual activity in females, as reported previously by Astal *et al.*[14] and Khalifa *et al.*[15].

Females of the reproductive age group (between 21 and 50 years) constituted 58.66% of total cases, compared to only 16.6% male patients. However, males (50 years or more) had a higher incidence of UTI (5.8%) compared to only 0.96% females of the same age group. Approximately same findings were reported by Akram *et al.*[16] who reported that, women of reproductive age group formed the main group of adult patients with UTI (42.34% of all UTI detected in women of age 21-50 years). UTIs were reported in 62.42% of females and in 37.67% of males. It has been extensively reported that adult women have a higher prevalence of UTI than men, principally owing to anatomic and physical factors.

This study showed that *E. coli* was the commonest pathogen causing UTI in 47 (45.19%) cases and it was similar to the findings of Mandal *et al*[13], Sharma and Paul[17].

The antimicrobial sensitivity pattern of the *E. coli* isolates in our study presents a disturbing pattern of antibiotic resistance, compared to previous studies done in India. The comparison of resistance patterns of uropathogenic *E. coli* in various studies is shown in Table 5.

Alarmingly, high level of drug resistance to first line of antibiotics was observed in our study, as all gram negative

Table 5: Comparison of resistance patterns of uropathogenic *E. coli* in recent studies from different cities of India

Authors	Place	Year	Antibiotic resistance (%)						
			AMP	COT	NIT	GEN	NOR	CE	IMP
Present study	Moradabad	2013	100	87.2	78.7	80.9	87.2	97.9	27.7
Dogra V *et al.*[18]	Delhi	2012	-	-	-	50.6	76.3	57.3	10.7
Sood and Gupta[19]	Jaipur	2012	81.8	67.8	5.7	28.2	77.88	70	-
Eshwarappa M *et al.*[20]	Bangalore	2011	-	33.5	28.6	49.2	74.1	-	3.9

AMP: Aminopenicillins, COT: Cotrimoxazole, NIT: Nitrofurantoin, GEN: Gentamicin, NOR: Norfloxacin, CE: 3rd gen. cephalosporins, IMP: Imipenem

isolates were resistant to ampicillin, while ampicillin/sulbactam was found to be sensitive in only 5 (10.6%) *E. coli* and 2 (10.5%) *Klebsiella* spp. isolates. *E. coli* and *Klebsiella* spp. isolates were also less sensitive to cotrimoxazole (12.8% and 10.5% respectively), nitrofurantoin (21.3% and 21% respectively), norfloxacin (12.8% and 21% respectively) and gentamicin (19.1% and 26.3% respectively). While for second-line antibiotics, *E. coli* was more resistant (69.5%) than *Klebsiella* spp. (46.1%) in this region. Indian isolates showed higher resistance against ampicillin and co-trimoxazole than the isolates from USA (39.1% and 18.6%)[21, 22].

Nitrofurantoin has been used for more than five decades for the treatment of uncomplicated cystitis and it was found to remain active against common uropathogens. Recent data suggests that nitrofurantoin has retained a good amount of sensitivity (90.98%), both against ESBL producers and non-ESBL producers[23]. In our study, nitrofurantoin was found to be sensitive in only *E. coli* and *Klebsiella* spp. isolates, at frequency of 21.3% and 21% respectively and we are first to report such high level of resistance to nitrofurantoin from India.

Carbapenems have a broad spectrum of antibacterial activity, and these are resistant to hydrolysis by most β-lactamases including extended spectrum β-lactamases (ESBL) and Amp C β-lactamases. Overall imipenem resistance was less common compared to meropenem, as only 27.7% isolates of *E. coli* and 50% of *Acinetobacter* spp. were resistant to impenem compared to 50% of *Acinetobacter* spp., 33.4% of *Pseudomonas* spp., 32.3% of *E. coli*, 31.6% of *Klebsiella* spp. and 25% of *Citrobacter* spp. isolates were resistant to meropenem.

Least resistance was observed against reserved category of drugs i.e. polymixin B and tigecycline; nevertheless, resistance to these drugs was observed in 6.4% and 10.6% of *E. coli* and 15.8% and 79% of *Klebsiella* spp. isolates respectively. This data is worrisome, as it hints towards emergence of pan-drug resistant common uropathogens.

Staphylococcus spp. was the third most common isolate. High level of resistance to ampicillin and methicillin was documented in 88.89% of isolates, followed by norfloxacin in 77.8%, cotrimoxazole and levofloxacin in 66.67%, ampicillin/sulbactum in 62.12%, gentamicin in 44.45% and clindamycin in 22.23% isolates. However, all isolates were sensitive to vancomycin, but a watchful vigilance is required for emergence of vancomycin resistance in view of recent reports of reduced susceptibility of *S. aureus* to vancomycin[24].

Enterococcus spp. was the fourth most common isolate. No isolate was sensitive to ampicillin but 55.55% isolates were sensitive to ampicillin/sulbactum followed by, 33.33% to levofloxacin and only 22.22% to gentamicin. All isolates were sensitive to vancomycin.

CONCLUSION

The susceptibility patterns seen in our study seem to suggest that slowly but steadily multi-drug resistance in common uropathogens is emerging at an alarming rate, making urine culture and sensitivity absolutely necessary, before initiation of antibiotic therapy in cases of suspected UTIs.

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