Research Article

Prevalence and antibiogram of bacterial pathogens causing urinary tract infection in a tertiary care hospital

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ABSTRACT

Background: Urinary tract infection (UTI) is one of the most frequently reported bacterial infections in the community coming second to respiratory tract infections. Empirical antibiotic therapy is usually applied in the management of UTIs, which has resulted in rapidly emerging antimicrobial resistance in hospitals and the community. **Objective:** The objective of this study was to study the prevalence and antibiogram of bacterial pathogens causing UTI in our hospital. **Materials and Methods:** A cross-sectional retrospective study was done among patients visiting a tertiary care hospital in North India, for February–March 2018. Clean catch midstream urine samples were taken from patients. Urine culture was done using cystine lactose electrolyte deficient agar medium. A modified semi-quantitative technique was employed for culture (standard wire loop method). Single species count of more than 10^5 organisms per ml of urine was considered as significant. Identification of the organism was done on the basis of colony morphology, motility testing, and biochemical tests using standard microbiological methods. Antibiotic susceptibility testing was done using Kirby-Bauer disc diffusion method. **Results:** Of the total 3172 samples - 341 (10.75%) were culture positive. Of these 341 culture-positive samples, the most commonly reported organism was *Escherichia coli* (53.9%), followed by *Klebsiella* spp. (24.9%). Among the Gram-positive organisms, only *Enterococcus* spp. was isolated in 10.8% of the total culture-positive samples. The Gram-negative isolates were found to be highly resistant to norfloxacin (nx) (56.3%) followed by cotrimoxazole (54.5%). Among Gram-positive isolates also, highest resistance was reported for nx (83.4%) and resistance to vancomycin was seen in only 5.4% of the cases. **Conclusion:** The study shows that Gram-negative organisms are the leading cause of UTIs among adult population and periodic monitoring needs to be done to keep their emerging resistance in check.

KEY WORDS: Antibiogram; Prevalence; Bacteria; Urinary Tract Infection

INTRODUCTION

Urinary tract infection (UTI) is one of the most frequently reported bacterial infections in the community coming second to respiratory tract infections. It accounts for approximately 1 million hospitalizations annually.[1] A major public health problem, UTIs are associated with morbidity and financial burden - accounting for the majority of the health-care cost among urological diseases, exceeding even that of chronic renal failure, renal dialysis, and renal transplantation included in the study.[2]

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Gram-negative bacteria such as *Escherichia coli*, *Klebsiella* species, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Acinetobacter* species cause most of the UTIs, and Gram-positive bacteria such as *Enterococcus* species and *Staphylococcus* species also contribute to causing UTIs.[3] *E. coli*, the commonest causative agent of the family *Enterobacteriacea*e, accounting for 75.0–90.0% of all UTIs in inmates and outpatients.[4]

Over the years, management of UTIs has been aided by the introduction of antimicrobial therapy, although empirical antibiotic therapy is usually applied in these cases.[2,4] As a result with current antibiotic therapies, antimicrobial resistance is rapidly emerging in hospitals and the community.[5] Treatment becomes even more challenging in the presence of risk factors such as old age, comorbidity, and immunocompromised state.[6]

Thus, knowledge of the common uropathogens and their susceptibility to commonly used antibiotics is the need of the hour.[4] Initial appropriate empirical treatment requires a good knowledge of epidemiological data.[7]

In this present study, the most recent epidemiological data of UTI from a tertiary care hospital in North India have been summarized.

MATERIALS AND METHODS

Study population: A cross-sectional retrospective study was done among patients visiting a tertiary care hospital in North India, for February–March 2018. A total of 3572 patients were included in the study. All adult patients who had a presumptive diagnosis of UTI and aged 16–80 years were included in the study.

Collection of Sample

All patients suspected of having UTI were instructed to give midstream, clean catch urine samples in a wide mouth sterile container. The sample was collected before starting the antibiotics. Urine samples were examined and processed for bacteriuria in the laboratory as soon as possible after collection.

Microscopy

Urine specimens were examined by wet mounts for the presence of any pus cells, microorganisms, red blood cells, cast and crystals, or any other findings.

Culture and identification of isolates a modified semi-quantitative technique were employed (standard wire loop method). A standard bacteriological loopful of urine (0.01 ml) was inoculated over the surface of cystine lactose electrolyte deficient agar plate. The plates were then incubated at 37°C for 18–24 h. Single species count of more than 10^5 organisms per ml of urine was considered as significant. Identification of the organism was done on the basis of colony morphology, motility testing, and biochemical tests using standard microbiological methods.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was done using Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines. The bacterial suspension was made by inoculating 4–5 isolated identical colonies in peptone water. The peptone water was then incubated at 37°C for 2 h. After 2 h of incubation, the turbidity was standardized using 0.5 McFarland standards and inoculated plates were incubated at 37°C. Test organisms were streaked on Muller–Hinton agar (MHA) plates using sterile swab. The appropriate antibiotic disc was then placed firmly onto the surface of the dried plates using sterile forceps, depending on whether the test organism plated was a Gram-negative or Gram-positive organism. The six antibiotic discs per plate were placed and plates were left at room temperature for 1 h to allow diffusion of the antibiotics from the disc into the medium. The plates were then incubated at 37°C for 18–24 h. The plates were read the next day, zone diameters were noted and interpreted as per CLSI guidelines 2016.

The following antibiotics were used: Nitrofurantoin (nit) (300 µg), amikacin (ak) (30 µg), cotrimoxazole (cot) (25 µg), gentamicin (10 µg), norfloxacin (nx) (10 µg), ertapenem (etp) (10 µg), piperacillin/tazobactam (pit) (100/10 µg), aztreonam (30 µg), ceftazidime (caz) (30 µg), cefazolin (30 µg), penicillin (10 U), vancomycin (30 µg), tetracycline (30 µg), erythromycin (15 µg), chloramphenicol (µg), high-level gentamicin (120 µg), and linezolid (30 µg).

Test for Extended-spectrum Beta-lactamases (ESBL) Production

Phenotypic confirmatory test with combination disc. For this test, a disc of caz 30 µ alone and a disc of caz plus clavulanic acid (30/10 µ) were used. The discs were placed at least 25 mm apart center-to-center on a lawn culture of test isolate on MHA plate and incubated overnight at 37°C. Difference in zone diameters with and without clavulanic acid was measured. Interpretation: An increase of greater than 5 mm in inhibition zone around combination disc of caz plus clavulanic acid disc versus the inhibition zone diameter around caz disc alone - confirms ESBL production.

RESULTS

Of the total 3172 samples, 65.1% were female and 34.9% were male patients. A single species count of more than 10^5 organisms per ml of urine was considered as significant. A total of 341 (10.75%) were culture positive - 70.3% of these organisms were isolated from female patients and 29.6% from male patients. Of these 341 culture-positive samples, the most commonly reported organism was *E. coli* accounting for 53.9% (n = 184) of the samples followed by *Klebsiella* spp. - 24.9% (n = 85). The other less commonly isolated organisms were - *Acinetobacter* spp. from 4.9%, *Proteus* spp. from 2.6%, *Pseudomonas* spp. from 1.7%, and *Citrobacter* spp. from 0.5% of the culture-positive samples. Among the Gram-positive organisms, only *Enterococcus* spp. was isolated in 10.8% (n = 37) of the total culture-positive samples [Table 1].

Antibiotic Susceptibility

Comparison of the susceptibility pattern on the basis of zone diameter (using Kirby-Bauer disc diffusion method) was done and has been illustrated in Table 2. Organism-wise pattern of resistance has been demonstrated in Tables 3 and 4.

Table 1: Pathogens isolated from urine samples

Organism	*n* (%)
E. coli	184 (53.9)
Klebsiella spp.	85 (24.9)
Acinetobacter spp.	17 (4.9)
Pseudomonas spp.	6 (1.7)
Proteus spp.	10 (2.9)
Citrobacter spp.	2 (0.02)
Enterococcus spp.	37 (10.8)
Total	341 (10.7)

E. coli: Escherichia coli

Table 2: Antibiotic susceptibility for the uropathogens isolated

Antibiotic	Number of resistant isolates (%)
ak	56 (16.4)
pit	57 (16.7)
nx	192 (56.3)
nit	45 (13.2)
caz	162 (47.5)
cf	161 (47.2)
cot	186 (54.5)
etp	29 (8.5)

ak: Amikacin, caz: Ceftazidime, cf: Cefazolin, nx: Norfloxacin, nit: Nitrofurantoin, cot: Cotrimoxazole, pit: Piperacillin-tazobactam, etp: Ertapenem, Cl: Colistin

The Gram-negative isolates were found to be highly resistant to nx (56.3%) followed by cot (54.5%). On the other hand, Gram-negative organisms were least resistant to etp (8.5%) followed by nit (13.2%) [Table 3]. Among Gram-positive isolates, the highest resistance was reported for nx (83.4%) followed by erythromycin (78.4%) and ak (67.5%). None of the isolates was resistant to linezolid. Resistance to vancomycin was seen in only 5.4% of the cases [Table 4].

ESBL production was found in 21.2% of the *E. coli* isolates and 12.9% of *Klebsiella* species to be ESBL producers.

DISCUSSION

The present study reports a prevalence rate of 10.7% among the patients suspected of having UTIs. Similar prevalence rates have been reported by Indian authors.[6-9] Eshwarappa *et al*.[6] did a study in South India and reported a prevalence rate of 9.17%. A study done by Arghya and Tuhina,[8] in 2015, in Varanasi, reported a prevalence rate of 9.77% and Akram *et al*.[9] reported a prevalence rate of 10.86% from Aligarh. A study done in Rajasthan by Sood and Gupta[2] reported a prevalence rate of 17.16%. However, some other studies done in India have reported higher prevalence rate of UTI. Reports from southern part of the country also showed higher prevalence rates of 22.78% and 32% by Murugan *et al*.[10] and Shanthi *et al*.,[1] respectively, while a study done in Northeast India showed a prevalence rate of 30%.[4] The high level of resistance in these studies could be because of geographical variation. Similar studies done outside India have reported a prevalence rate of 20.69% in Tehran[11] and 22.7% in Ethiopia.[12]

Of the culture positive 341 samples - 70.3% of these organisms were isolated from female patients and 29.6% from male patients. This correlates to the studies of Shanthi

Table 3: Antibiotic susceptibility pattern for Gram-negative bacilli

Antimicrobial	*E. coli* *n*=184 (%)	*Klebsiella* *n*=85 (%)	*Acinea* *n*=17 (%)	*Psd* *n*=6 (%)	*Proteus* *n*=10 (%)	*Citrobacter* *n*=2 (%)
ak	25 (13.6)	17 (20.0)	7 (41.2)	2 (33.3)	5 (50)	0
pit	29 (15.8)	21 (24.7)	5 (29.4)	1 (16.7)	1 (10)	0
nx	129 (70.1)	41 (48.2)	11 (64.7)	2 (33.3)	7 (70)	1(5)
nit	10 (5.4)	9 (10.6)	13 (76.5)	3 (50.0)	10 (100)	0
caz	96 (52.2)	45 (52.9)	13 (76.5)	2 (33.3)	6 (60)	0
cf	97 (52.7)	40 (47.1)	13 (76.5)	3 (50.0)	7 (70)	1 (50)
cot	124 (67.4)	39 (45.9)	12 (70.6)	2 (33.3)	8 (80)	1 (50)
etp	18 (9.8)	13 (15.3)	5 (29.4)	2 (33.3)	1 (10)	0
cl	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (100)	0
pb	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0)	0
azt	0 (0.0) 0	0 (0.0)	0 (0.0)	0.0	0 (0)	0
Total	184	85 (100.0)	17 (100.0)	6	10	2

ak: Amikacin, caz: Ceftazidime, cf: Cefazolin, nx: Norfloxacin, nit: Nitrofurantoin, cot: Cotrimoxazole, pit: Piperacillin-tazobactam, etp: Ertapenem, Cl: Colistin

Table 4: Antibiotic susceptibility pattern for Gram-positive cocci

Organism	ery	nx	nit	pen	lz	va	tet	hlg	ak
Enterococcus spp. *n*=37 (%)	29 (78.4)	31 (83.4)	2 (5.4)	13 (35.1)	0 (0)	2 (5.4)	16 (43.2)	21 (56.7)	25 (67.5)

ery: Erythromycin, pen: Penicillin, nx: Norfloxacin, nit: Nitrofurantoin, lz: Linezolid, va: Vancomycin, tet: Tetracycline, hlg: High-level gentamicin, ak: Amikacin

et al.,[1] Gupta *et al*.,[13] and Akram *et al*.[9] This could be due to factors such as length of urethra, distance of urogenital meatus from anus, and the antibacterial properties of prostatic fluid.[1]

The most common organism isolated in our study was *E. coli* (53.9%) followed by *Klebsiella* spp. (24.9%). This finding was in agreement with studies done by Indian authors.[1,4,9,14] *Acinetobacter* spp. was isolated in 4.9% of cases in our study, which was similar to other studies done in India.[1,9] *Pseudomonas* spp. accounted for 1.7% of the total cases in our study which was lower as compared to 4.9% as reported from Northeast India by Chongtham *et al*.[4] and 3.75% as reported from South India by Shanthi *et al*.[1] In the present study, among the Gram-positive organisms, only *Enterococcus* spp. was isolated - accounting for 10.8% of the total isolates. Similar rate was reported from Imphal by Chongtham *et al*. (9.12%) while the study from Varanasi done by Arghya and Tuhina reported a higher prevalence rate (21.79%).[4,8]

Overall, resistance among the isolates was maximum for nx (56.3%) followed by cot (54.5%). This could be because of frequent prescription of these drugs as the first-line treatment of UTI in the hospital. Similar results were reported by Chongtham *et al*. and Shanthi *et al*.[1,4] A generalized reduction in bacterial susceptibility toward quinolones has been observed which could be because it is one of the drugs of choice for the treatment of UTI.[4] This finding was also consistent with a study done in Karnataka by Eswarappa M *et al*. who reported a high rate of resistance against quinolones.[6]

Resistance to cephalosporins was seen in 47.3–47.5% of the isolates. Only 16.4% of the isolates were resistant to ak; this was contradictory to the findings of Akram *et al*.[9] where 76% of the isolates were resistant to ak. Other studies, however, have shown similar sensitivity pattern - Pandey *et al*.[3] from Nepal reported 20% of the isolates were resistant to ak. According to a study done by Chongtham *et al*.,[4] the most sensitive antibiotic for *E. coli* and *Klebsiella* spp. was aminoglycosides. In the present study, ak was found to be more sensitive among the Gram-negative isolates than Gram-positive cocci (67.5% vs. 16.4%). This was in agreement with a study done in Gujarat by Devmurari *et al*.[15]

Resistance among the Gram-negative isolates was least in case of carbapenem (8.5%). This was in accordance with a study done by Devmurari *et al*.[15] However, some other studies have reported lower resistance rates.[6,16] Although the rate of resistance against carbapenems is reportedly low at present, it raises concern over which options to choose for the treatment of drug-resistant cases. Some strains have now developed various effective means to deal with the carbapenems. Multiple mechanisms such as - production of beta-lactamases which destroy the antibiotics, blocking the entry of these antibiotics, or by efflux pumps which actively pump out these antibiotics, have led to rise in the resistance against carbapenems.[17]

Of the total *E. coli* and *Klebsiella* spp. Isolates, 5.4% and 10.7%%, respectively, showed resistance to nit, which could be because of less use of the drug to treat UTI in the region. However, *E. coli* and *Klebsiella* isolates were highly resistant against nx (70.1% and 48.2% resistant, respectively). Many studies worldwide have also reported a sharp increase in quinolone-resistant *E. coli* isolates from UTIs. The prevalence of quinolone resistance in Bangladesh was 26% (Kahlmeter *et al*.). Increased resistance for nx in *E. coli* has also been reported by Shanthi *et al*.[1] from South India.

Resistance rate of cot which is commonly used in UTI was high with 67.4% of *E. coli*, 45.9% of *Klebsiella* spp. being resistant to it, similar rates were also reported by other studies.[1,9] Overall, resistance rate among Gram-negative isolates (54.5%) showed higher resistance against co-trimoxazole than the isolates from the USA[18] (18.6%) and Europe[19] (14.1%). On the other hand, a comparable rate of resistance against this antibiotic was reported from countries such as Senegal (55%) and Taiwan (56%) is comparable with Indian isolates.[9]

The present study revealed 21.2% of the *E. coli* isolates and 12.9% of Klebsiella species to be ESBL producers. Similar finding was reported from Rajasthan by Sood and Gupta, and Aggarwal *et al*. reported 40% of *E. coli* and 54.54% of *Klebsiella* species to be ESBL producers from Rohtak, Haryana.[20] In another study from Nagpur, 18.5% of *E. coli* isolates and 25.6% of *Klebsiella* isolates were found to be ESBL producers.[21] High resistance was noted among *Acinetobacter* spp. for nit, nx, cephalosporins, and cot. However, a study done in Aligarh by Akram *et al*. reported highest percent susceptibility (100%) against nx for *Acinetobacter* spp.

An overall 5.4% resistance for vancomycin was observed in Gram-positive cocci belonging to the *Enterococcus* species, whereas other studies have reported much lower resistance rate.[2,14] Resistance to ak was reported among 83.4% of *Enterococcus* spp., whereas a study done in North India by Vohra *et al*.[14] reported 50% resistance for ak. Only 5.4%

of the *Enterococcus* spp. were found to be resistant to nit contrary to a study done by Vohra *et al*.[14] that reported a high (62.5%) resistance to nit.

Our study points out emerging high resistance rate among UTI patients. This was in agreement with other studies done in India.[1,2,6,9] Fluoroquinolones are prescribed in a wide variety of infections, they are capable of permeating most body compartments accounting for the emergence of their resistance.[2] Similar high resistance rate has been reported for cot in the present study. Thus, the present study highlights the need for urgent measures to counteract increased resistance to these drugs, or they need to be judiciously prescribed in uncomplicated infections.

CONCLUSION

The study shows that Gram-negative organisms are the leading cause of UTIs among adult population and they have developed resistance mechanisms against the routinely prescribed drugs. Their distribution and resistance pattern varies with geographical and demographic factors. Periodic monitoring and surveillance need to be done to keep the emerging resistance among uropathogens in check so that more definitive treatment can be given to the patient.

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