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Original Research Article

Uropathogens and their antibiotic susceptibility profile in a tertiary care hospital

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ABSTRACT

Background: Urinary tract infections (UTI) are one of the most common infections in the community and hospitals. Uropathogens colonize the urinary tract and may ascend to bladder causing cystitis, if left untreated reach kidneys through ureters can be responsible for acute pyelonephritis and cause renal damage.
Aims: The aim of the present study is to determine the prevalence of urinary tract infections and antibiotic susceptibility pattern in a tertiary care hospital.
Settings & Design: This is an observational study conducted in Microbiology department, Hind Institute of Medical Sciences, Barabanki.
Materials and Methods: A total of 623 urine(mid-stream) samples were collected from indoor and outdoor departments of hospital and culture was done on UTI chromogenic agar using semiquantitative method. Antibiotic sensitivity test was performed using Kirby Bauer disc diffusion method.
Results: Prevalence of urinary tract infections is 29% in the study. *Escherichia coli* (43%) is the most common micro-organism isolated followed by *Enterococcus* (13%), *Staphylococcus aureus* (11%), *Acinetobacter* (10.4%), *Klebsiella* (8.8%), *Pseudomonas* (3.3%), *Proteus*(1.6%), CONS & *Citrobacter*(1.1%) and *Candida*(6.6%). The females(56.6%) are more commonly affected than males.
Conclusion: In the present study, beta-lactamase inhibitors and aminoglycosides were effective drugs against gram negative bacteria. Vancomycin and linezolid were sensitive in gram positive bacteria. Nitrofurantoin is the promising drug in cases of uncomplicated UTI, and safe to use in pregnancy. High recurrence rates and antimicrobial resistance are responsible for increasing the burden of disease. It is advised to use the antibiotics judiciously as per the hospital antibiotic policy which will help prevent multidrug resistance micro-organism further reducing morbidity and mortality.

1. Introduction

Urinary tract infection (UTI) is the common infection of urinary tract affecting all age groups of population. Various micro-organisms are responsible for UTI like bacteria, viruses, fungus, parasite. Most common infection is caused by bacteria. The causative micro-organisms like *Escherichia coli, Klebsiella, Proteus*, *Pseudomonas, Citrobacter, Acinetobacter*, *Enterococcus and Staphylococcus aureus are* responsible for UTI.[1,2]

Uropathogens vary from place to place depending on demographic features, community or hospital based, use of different antibiotics in different hospital settings also affects the antimicrobial sensitivity profile depending upon the use of broad-spectrum antibiotics.[1,3] There are various factors responsible for the virulence mechanism of uropathogens to enter the urinary tract and cause infection. *Enterobacteriaceae* family especially Uropathogenic *Escherichia coli* (UPEC) is frequently associated with UTI. It attaches to the uroepithelium by pili, Type 1 fimbriae, P fimbriae and adhesions promoting bacterial colonisation and causing inflammatory response in host.[4–6]

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Certain anatomical and physiological features play an important role in urinary tract infections. In females due to the short urethra, faecal contamination of the vaginal orifice is frequently leading to UTI.[1,2] It is more prevalent in females mainly sexually active young women, long term catheterized patients and elderly male individuals.[7] In elderly male individuals due to conditions like neurogenic bladder, prostate enlargement, there is incomplete emptying of bladder leading to residual urine. The vesico-ureteric reflux is common in pregnancy leading to recurrence.[1,4] Recurrence is common in all such individuals due to the predisposing factors. It is found that about 50% of the females develop urinary tract infection at least once in their lifetime.[1,7]

The predisposing factors responsible for UTI should be identified early and treated in time. Delayed treatment can lead to recurrence and if persist for long time can cause renal complications like hydronephrosis, acute pyelonephritis, acute renal failure, and irreversible kidney damage.[1,5] The aim of the present study is to know the prevalence of urinary tract infection, their causative micro-organisms(uropathogens) and antibiotic susceptibility pattern in our tertiary care hospital setup.

2. Materials and Methods

The present study is an observational study to determine the prevalence of bacteria causing urinary tract infections (Uropathogens) and their antimicrobial sensitivity profile carried out in Bacteriology laboratory in the department of Microbiology, Hind Institute of Medical Sciences, Barabanki. The duration of the study was six months from 1^{st} January 2019 to 30 June 2019. A total of 673 samples with symptoms of urinary tract infection were included in the study both indoor and outdoor location of various departments of the hospital.

2.1. Ethical clearence

Patient's consent was taken on an Informed Consent form. The study was ethically approved by the Institutional Ethical Committee (IEC).

2.2. Inclusion criteria

1. Patients with history of increased frequency, urgency, dysuria, suprapubic tenderness, and fever.
2. No history of antibiotics intake within one month.

2.3. Exclusion criteria

1. Patients with underlying chronic renal disease.
2. Patients on antibiotics.

2.4. Sample collection and transport

A total of 673 non duplicate urine samples were taken both from outpatient departments and various wards. The mid-stream urine (MSU) was collected in a sterile wide-mouthed screw-capped container. The first part of the urine was not to be collected (as it might contain commensals from the anterior urethral region). The samples collected were transported to the laboratory, and if there was a delay, the samples were stored at 4^{o}C till further processing.

2.5. Sample processing

In the laboratory, uncentrifuged urine sample was observed in 400x magnification for epithelial cells, pus cells, RBCs and any micro-organisms. Culture of the urine samples collected was performed with standard calibrated loop on UTI Chromogenic agar (HI media-code M1353R). The inoculated plates were incubated at 37^{o}C for 18-24hrs under aerobic conditions.

2.6. Bacterial identification

The micro-organisms were identified on the culture media on the basis of colony morphology with different colors i.e., *Escherichia coli* – purple-colored colonies, *Klebsiella* –blue mucoid colonies, *Acinetobacter* – pale-white colonies, *Staphylococcus aureus* - golden yellow colonies and *Enterococcus* - blue -green colored, small colonies. Further species identification was done with the help of biochemical reactions as per standard methodology.[8]

2.7. Interpretation was done based on semi-quantitative analysis as

1. Significant growth
2. Non-significant growth
3. Contamination
4. Sterile

2.8. Antibiotic susceptibility testing (AST)

Antibiotic Susceptibility Testing (AST) was done for culture plates with significant growth. The isolated colonies from the identified species were suspended in normal saline and the inoculum was prepared. The density of the suspension was compared with the 0.5 Mac Farland's opacity standards. Kirby Bauer's disc diffusion method was performed on Muller Hinton agar (HI media).The results were interpretated using the Clinical Laboratory Standards Institute 2018 guidelines.[9]

The antibiotics tested for Gram negative bacteria (potency in μg/disc) (Himedia) were as follows-ampicillin (10), gentamicin (10), amikacin (10), ceftazidime (30), ceftriaxone(30), cefepime(30), amoxicillin-clavulanate (20/10), piperacillin-tazobactam (100/10), ampicillin-sulbactam(10/10), ciprofloxacin (5), levofloxacin (5),

norfloxacin (10), ertapenem (10), imipenem (10), meropenem (10), nitrofurantoin (300). The antibiotics tested for Gram positive bacteria (potency in μg/disc) (Himedia) were as follows- penicillin (10U), cefoxitin (30), vancomycin (30), linezolid (30). High level gentamicin (120) was used only for *Enterococcus* spp. Novobiocin (30) is used in Coagulase negative *Staphyloccoccus* species to differentiate between *S. saprophyticus* and *S. epidermidis*.

2.9. Statistical analysis

Data was collected on a preformed questionnaire containing personal details,clinical and demographic details of patients.The collected data was entered in an Excel sheet and graphs were generated. The results were statistically analysed using Advanced excel software and presented in the form of tables and figures.

3. Results

Out of the 623 non-duplicate urine samples collected to study the prevalence of urinary tract infection (UTI), significant bacteriuria ($\geq 10^5$ CFU) was seen in 181 samples (29%). 341 samples (54.7%) were sterile, while insignificant growth with bacterial count of $< 10^5$CFU/ml was found in 87 samples (13.9%) and 14 sample (2.2%) were contaminated. Out of the total samples collected, 429 (68.8%) samples were collected from OPD patients, and 194 (31.1%) samples were from patients admitted in wards as depicted in Table 1.

The total number of Gram-negative bacteria among the isolates were 124 (68.5%), whereas Gram-positive isolates were 45 (24.9%), and 12 (6.6%) isolates were *Candida* , as shown in Figure 1. Out of the total culture positive samples, 102 females (56.3%) were culture positive compared to 79 males (43.6%) as described in Table 2.

Escherichia coli(n=78) was the most common causative organism with 43%, followed by *Enterococcus faecalis*(n=23) with 13%, followed by *Staphylococcus aureus*(n=20) with 11%, 19 *Acinetobacter spp.*(10.4%), 16 *Klebsiella spp.* (8.8%), 6 *Pseudomonas spp*(3.3%), 3 *Proteus spp* (1.6%), 2 Coagulase negative *Staphylococcus spp.* (1.1%), 2 *Citrobacter spp* (1.1%) and *Candida spp*(6.6%).[Figure 2] Antibiotic sensitivity profile of gram negative bacteria (*Enterobacteriaceae*) and non fermenters are depicted in Tables 3 and 4. Antibiotic sensitivity profile of gram positive bacteria is shown in Table 5.

4. Discussion

In the present study, the prevalence of urinary tract infection is 29% which is like the studies conducted with 30%[10] in South India, 32.6%[1] in Saudi Arabia and 36.1%[11] in Pakistan. The relatively lower prevalence was seen in studies with 6.3%[12] in Tehran, 9.7%[3] in Himachal,10.25%[13] in Delhi, 10.8%[14] in Aligarh, 12.2%[15] in Port Blair, 17.19%[4] in Jaipur. Higher prevalence is found in Kolkata with 55%[16] and 53.8%[17] in Meerut.

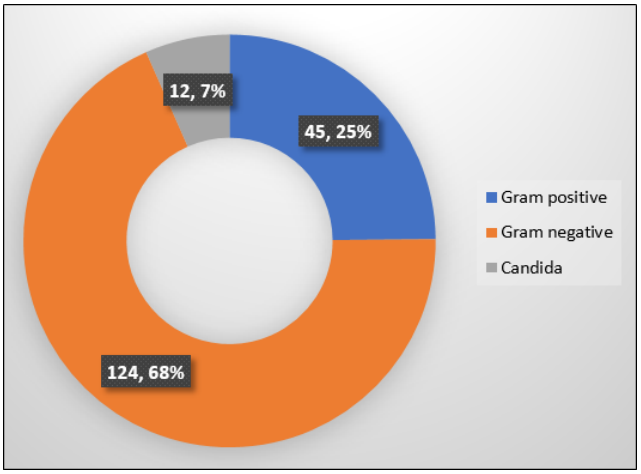

Fig. 1: Distribution of different isolates on the basis of morphology

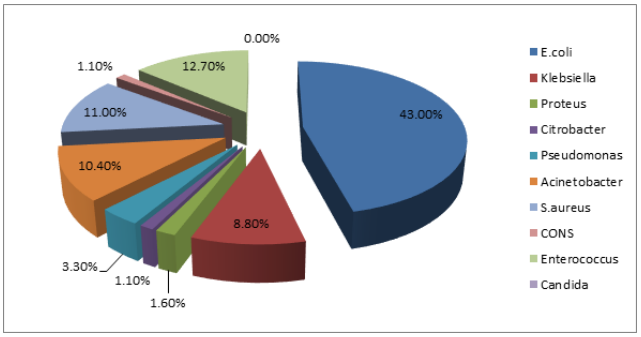

Fig. 2: Distribution of micro-organisms causing urinary tract infection

The most common micro-organism isolated is *Escherichia coli* (43%) which is concordant with the other studies conducted with 36%,[16] 42.6%,[17] 48.3%,[10] 59.2%,[18] 59.8%,[3] 61.8%,[4] 70.9%[13] from various parts of India supporting our findings. From outside India, also 27%,[1] 41.9%,[19] 68.3%.[11]

Urinary tract infection is more common in females (56.6%) compared to males. Our findings are in support of other studies with 45.2%,[2] 50.7%,[11] 58.5%,[18] 62.4%,[4] 65.3%,[15] 73%,[1] 73.6%.[17] The reason may be structural anatomy of female genital tract where uropathogens ascend to bladder with short urethra having close proximity to anal orifice, whereas in males greater length of urethra is surrounded by antibacterial prostatic fluid and dried environment.[15,17,20]

Escherichia coli is followed by *Enterococcus spp* (13%) isolates similar to the studies conducted with 9.2%,[4] 9.7%[2] and 10.1%.[18] Both these organisms are of faecal origin so found responsible to colonize urinary tract and cause infection. In a study,[3] *Enterococcus* spp. is the third

Table 1: Distribution of total samples

Variables	Number of isolates	% of isolates
Growth in culture		
Significant growth	181	29%
Non-significant	87	13.9%
Sterile	341	54.7%
Contamination	14	2.2%
Location		
Indoor	429	68.8%
Outdoor	194	31.1%
Total	623	100%

Table 2: Distribution of culture positive isolates

Gender	Number of isolates	% of isolates
Female	102	56.3%
Male	79	43.7%
Total	181	100%

Table 3: Antibiotic sensitivity profile for gram negative bacteria (*Enterobacteriaceae*)

Drugs	E. coli (n=78)	Klebsiella spp (n=16)	Citrobacter spp (n=2)	Proteus spp (n=3)
Ampicillin	25(32%)	5(31.5%)	0(0%)	3(100%)
Ceftriaxone	34(43.5%)	7(43.7%)	1(50%)	2(66.7%)
Ceftazidime	34(43.5%)	7(43.7%)	1(50%)	2(66.7%)
Cefepime	34(43.5%)	7(43.7%)	1(50%)	2(66.7%)
Amoxicillin-clavulanate	73(93.5%)	16(100%)	2(100%)	3(100%)
Piperacillin-tazobactam	73(93.5%)	15(93.7%)	2(100%)	3(100%)
Gentamicin	58(74.3%)	11(68.7%)	1(50%)	2(66.7%)
Amikacin	73(93.5%)	14(87.5%)	1(50%)	2(66.7%)
Ciprofloxacin	32(41%)	7(43.7%)	1(50%)	1(33.3%)
Levofloxacin	73(93.5%)	14(87.5%)	2(100%)	3(100%)
Norfloxacin	25(32%)	8(50%)	0(0%)	0(0%)
Nitrofurantoin	77(98.7%)	13(81.2%	2(100%)	-
Imipenem	67(85.8%)	13(81.2%)	1(50%)	1(33.3%)
Meropenem	78(100%)	16(100%)	1(50%)	3(100%)
Ertapenem	73(93.5%)	16(100%)	1(50%)	3(100%)

Table 4: Antibiotic sensitivity profile for gram negative bacteria (Non fermenters)

Drugs	Pseudomonas spp (n=6)	Acinetobacter spp (n=19)
Ampicillin-sulbactam	-	11(58%)
Ceftriaxone	-	2(10.5%)
Ceftazidime	4(66.7%)	2(10.5%)
Cefepime	5(83%)	5(26.3%)
Piperacillin-tazobactam	5(83.3%)	13(68.4%)
Gentamicin	5(83.3%)	9(47%)
Amikacin	5(83.3%)	11(57.9%)
Ciprofloxacin	4(66.7%)	8(42.1%)
Levofloxacin	6(100%)	17(89.5%)
Imipenem	5(83.3%)	16(84.2%)
Meropenem	6(100%)	17(89.5%)

Table 5: Antibiotic sensitivity profile for gram positive bacteria

Drugs	Staphylococcus aureus (n=20)	CONS (n=2)	Enterococcus (n=23)
Penicillin	6(30%)	1(50%)	12(52%)
Ampicillin	20(100%)	2(100%)	15(65%)
Cefoxitin	10(50%)	1(50%)	-
Erythromycin	19(95%)	2(100%)	23(100%)
Clindamycin	19(95%)	2(100%)	-
Gentamicin	17(85%)	2(100%)	-
High level gentamicin	-	-	8(34.7%)
Ciprofloxacin	15(75%)	1(50%)	13(56.5%)
Levofloxacin	19(95%)	2(100%)	17(73.9%)
Norfloxacin	11(55%)	1(50%)	20(86.9%)
Nitrofurantoin	19(95%)	2(100%)	21(91.3%)
Vancomycin	20(100%)	2(100%)	23(100%)
Linezolid	20(100%)	2(100%)	23(100%)

common micro-organism with 8.7% isolates. Some studies reported *Klebsiella* as the second common isolates with 9.3%[11] and 9.7%[4] prevalence whereas our study reported *Klebsiella* (8.8%) as the third common micro-organism after *Enterococcus* spp (13%).

Staphylococcus aureus accounts for 11% prevalence in our study whereas other studies reported 3.3% isolates.[3,13] Some studies reported higher prevalence of 31.4%.[19] *Proteus* (1.6%) and *Citrobacter* (1.1%) are the least common pathogens reported in our study. Other studies also reported nearly 2% *Proteus* isolates[3,11,13] and 2% *Citrobacter* isolates.[3] We have reported only 1.1% Coagulase negative *Staphylococcus* in the study but it is an important micro-organism found in patients with chronic indwelling urethral catheters.[21] The present study reported 6.6% *Candida spp* whereas another study reported 2.7% isolates causing urinary tract infections.[3]

In the present study, there are 54.7% *Enterobacteriaceae*,13.8% non-fermenters and 24.8%-gram positive bacteria. Another study reported similar prevalence with *Enterobacteriaceae* (76.8%), non-fermenters (5.5%) and gram positive bacteria (14.2%).[4] In Mehrishi et al., similar findings were found with *Enterobacteriaceae* (67%), non-fermenters (9.6%) and gram positive bacteria(18%).[3]

Among non-fermenters, 3.3% *Pseudomonas spp* were reported in our study similar to other studies with 4.1%,[11] 5.4%[3] and 9.3%[18] whereas we reported 10.4% *Acinetobacter spp* in our study which is higher than other studies with 0.98%[13] and 1.6%[3] isolates.

In our study, *Enterobacteriaceae* showed 80-100% sensitivity to carbapenems like meropenem, imipenem and ertapenem. Among the cephalosporins and beta lactam group of antibiotics, there is nearly 50% resistance and among fluoroquinolones, ciprofloxacin and norfloxacin showed up to 70% resistance whereas levofloxacin shows 90% sensitivity. It has been found that beta-lactamase inhibitors like amoxicillin-clavulanate and piperacillin-tazobactam have 90% sensitivity to *Enterobacteriaceae* members. Nitrofurantoin, the drug of urinary route has good sensitivity for *Escherichia coli* (98.7%). Among aminoglycosides, gentamicin showed 50% sensitivity whereas amikacin is more sensitive (90%).

Our study is supported by the findings of other studies where high resistance is seen in cephalosporins, beta-lactams group of antibiotics, fluoroquinolones to *Enterobacteriaceae* whereas high sensitivity to Nitrofurantoin, amikacin and carbapenems.[2,3] In another study, carbapenems and beta-lactamase inhibitors are the promising drugs along with Nitrofurantoin with high sensitivity whereas low sensitivity to fluoroquinolones like ciprofloxacin and norfloxacin(98%).[10] It has been suggested that such high resistance of fluroquinolones and beta-lactams group of antibiotics are responsible for multidrug resistant (MDR) which leads to high selection rate of Extended spectrum beta-lactamase (ESBL) producing micro-organisms.[10,22,23] In studies, similar drug resistance pattern was seen in *Escherichia coli* isolates with high sensitivity to Nitrofurantoin and Beta-lactamase inhibitors.[4,13,16,22]

It has been suggested by various studies that Nitrofurantoin is the most recommended drug for patients of UTI. In pregnancy, *Escherichia coli* is the most common causative agent for urinary tract infections with high resistance to beta-lactams group and fluroquinolones. Nitrofurantoin is also safe to be administered in all trimesters of pregnancy and considered as the drug of choice in UTI among pregnant females.[24,25]

Among non-fermenter gram negative bacteria, the present study reported high resistance to third generation cephalosporins (80%), aminoglycosides are moderately sensitive (50-70%) along with fluoroquinolones and amoxicillin-sulbactam whereas high sensitivity to carbapenems (90%). Another study reported high resistance

to amoxicillin-sulbactam contrary to our study but other drugs sensitivity pattern for non-fermenters corroborates with our study.

Among gram positive cocci, vancomycin and linezolid showed 100% sensitivity as found in other studies.[3] Our study showed high sensitivity to erythromycin, clindamycin and nitrofurantoin (80-100%), moderate sensitivity to fluoroquinolones like ciprofloxacin, norfloxacin and levofloxacin (50-75%) whereas low sensitivity to penicillin and ampicillin (30-50%). In *Staphylococcus aureus* and CONS, gentamicin showed high sensitivity (85-100%) and cefoxitin showed 50% sensitivity. In *Enterococcus spp*, high level gentamicin was 35% sensitive. Similar sensitivity profile was seen among gram positive bacteria in a study which supports our findings.[3]

5. Conclusion

Earlier diagnosis of uncomplicated UTI by symptoms can be controlled by antibiotics without further complications. It has been found that urine culture is usually sent after treatment failure or recurrence of urinary tract infection. The measures should be taken to encourage the collection of urine sample for culture before the start of empirical treatment which should be replaced by specific treatment after the sensitivity report. This study helps to generate antibiotic policy in hospital setup and empirical treatment can be planned accordingly to prevent the indiscriminate use of antibiotics. Hence, prevent multidrug resistance (MDR) micro-organisms to develop which can lead to morbidity and mortality. Awareness should be created among population especially females to maintain personal and environmental hygiene to prevent faecal contamination of urinary tract thus reducing the infection rate and promoting health.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare that there is no conflict of interest.

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