 DOI: 10.18231/2455-6807.2017.0005

Prevalence and Antibiogram of Uropathogens from Patients Attending Tertiary Care Hospital: An overview

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Introduction

Urinary tract infection (UTI) is one of the most important causes of morbidity in the general population, and is the second most common cause of hospital visits. Urinary tract infections are among the most common bacterial infections and account for a significant part of the workload in clinical microbiology laboratories.(1)

UTI refer to the presence of microbial pathogens within the urinary tract and it is usually classified by the infection site:- bladder [cystitis], kidney [pyelonephritis], or urine [bacteriuria] and also can be asymptomatic or symptomatic, UTIs that occur in a normal genitourinary tract with no prior instrumentation are considered as uncomplicated, whereas complicated infections are diagnosed in genitourinary tracts that have structural or functional abnormalities, including instrumentation such as indwelling urethral catheters, and are frequently asymptomatic.(2,3)

UTIs are caused by pathogenic invasion of the urinary tract which leads to an inflammatory response of the uroepithelium. Proliferation of bacteria in the urinary tract is the cause of urinary tract infection. The common etiologic agents of UTI includes *E. coli, Klebsiella spp, Proteus spp, Pseudomonas spp* as well as Gram positive organisms like Staphylococci and Enterococci.(4)

Treatment of UTI cases is often started empirically and therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens.(5) In spite of the availability and use of the antimicrobial drugs, UTIs caused by bacteria have been showing increasing trends in recent years. Much of the increase has been related to emerging antibiotic resistance in urinary tract pathogens.(6) The prevalence of antimicrobial resistance in urinary pathogens is increasing worldwide. Accurate bacteriologic records of culture results may provide guidance on empirical therapy before sensitivity patterns are available.(5,6) So the present study was undertaken to determine the prevalence & common bacteria causing UTI and antibiogram of isolated pathogens in our tertiary care hospital

Materials and Methods

Study Design: The present study is prospective type of study and was carried out at Department of Microbiology, Indian Institute of Medical Sciences Badnapur, Jalna, Maharashtra after approval from institutional ethics committee.

Patient Inclusion Criteria: The study included patients, both from OPD & IPD suspected of urinary tract infection, during the study period of January 2016 to December 2016.

Consent: A due written consent was obtained from the patient before proceeding with history, examination or sample collection.

History: A brief clinical history was obtained from each patient to find out if there is any risk factor predisposing to urinary tract infection.

Specimen Collection: A clean voided early morning midstream urine specimens was collected in a sterile container after proper aseptic precautions, before starting antibiotics. Diagnostic catheterization for urine sample collection was avoided as even under ideal conditions, catheterization itself leads to UTI in about 2% patients.

Processing in Laboratory:(7) Urine samples were examined & processed in the laboratory as soon as possible after collection. In case of delay, the sample was stored in a refrigerator.

Microscopy: Urine specimens were examined by wet mounts. Presence of any pus cells, micro-organisms, RBCs, cast and crystals or any other findings were noted.

Culture: A Urine specimen was cultured on Blood agar and MacConkey agar using 0.01 mm calibrated loop for semi-quantitative method. These plates were then incubated overnight in an incubator at 37^0c, and observed for growth.

Significant Colony(5,6,7)

- Counts $>10^5$ CFU/ml in midstream urine sample in a patients with no risk factors.
- $>10^3$ CFU/ml in midstream urine sample in a symptomatic patients or in a pregnant female

Identification of the organism (Isolate): The isolate was identified on the basis of Colony morphology, Motility testing, Biochemical tests using standard microbiological methods.(7)

Antibiotic Sensitivity Testing: It was carried out for commonly used antibiotics on Muller Hinton agar plate by standard Kirby Bauer disk diffusion method as per as CLSI guidelines.(8) The bacterial suspension was

made by inoculating 4-5 isolated identical colonies in peptone water.

After 2 hours of incubation, the turbidity was standardized by using 0.5 Mc Farland standards. By using sterile swab, a lawn culture was made on the Mueller-Hinton agar plates. The 6 antibiotic discs per plate were placed and inoculated plates were incubated at 37^0c .The results was read after overnight incubation and compared with the standard chart.

The following antibiotics were used: Nitrofurantoin (300µg), Amikacin (30µg), Cotrimoxazole (25µg), Gentamicin (10µg), Ciprofloxacin (5µg), Norfloxacin (10µg), Ofloxacin (5 µg), Ampicillin (10µg), Imipenem (10µg), Cefoxitin (30 µg), Pipercillin/Tazobactam (100/10µg), Aztreonam (30 µg), Ceftazidime (30 µg), Amoxyclav (20/10 µg), Cefuroxime (30 µg), Vancomycin (30 µg) and Ampicillin/Sulbactam (10/10µg).

Staphylococcus aureus (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) was used as quality control throughout the study for culture and antimicrobial susceptibility testing.

Statistical analysis: The results were expressed as percentages for analysis of various epidemiological details and for analysing the distribution of different bacterial isolates and their sensitivity pattern. Microsoft excel was used for the interpretation of these results.

Results

Out of 1895 consecutive urine samples received during the study period, 903 (47.65%) were sterile, 685(36.14%) showed significant growth, 58 (3.06%) showed insignificant growth and 249(13.13%) were found contaminated. 511(74.59%) samples were from female patients whereas male compromises for 175 (25.40%) samples. The highest isolation was found in (40.4%) age group of 21-40 years followed by (37.08%) 61-75 age group.

Of all the positive samples 381 samples were from hospitalized patients while 304 samples were from patients attending outpatient department. Details about different host characteristics are shown in Table 1,2,3,4.

Table 1: Total Number of Samples n= 1895

Samples	Percentage(%)
Positive samples (685)	36.14
Negative samples (1210)	63.86
Total (1895)	100

Table 2: Sex wise distribution n=685

Sex	Number (%)
Male	174 (25.40)
Female	511 (74.59)

Table 3: Age group wise distribution n=685

Age group	Numbers	Percentage(%)
< 1 yrs	15	2.1
1-20yrs	51	7.44
21-40yrs	277	40.4
41-60 yrs	88	12.8
60-80yrs	254	37.08
Total	685	100

Table 4: Department wise distribution n=685

Department	Number of cases	Percentage (%)
Medicine	146	21.31
Surgery	239	34.89
Intensive care unit	171	24.96
Paediatrics	23	3.35
OBGY	106	15.47
Total	685	100

Of the 685 culture positives, isolation of gram negative bacteria 613(89.48%) was more than gram positive bacteria 72(10.51%). *E.Coli* 411(60%) was the most commonly isolated pathogen irrespective of age group and departments followed by *Klebsiella* spp 192(28%), *Staphylococcus aureus 68*(10%), *Pseudomonas aeruginosa* 7(1.20%), *Enterococci* spp 4(0.5%), *Proteus* spp and *Acinetobacter* spp 4(0.5%).

The detailed microbiological data of pathogens and their antimicrobial susceptibility causing urinary tract infection are shown in Table 5, 6 & 7.

Table 5: Distribution of organisms isolated from urine sample

Name of organism	Numbers (n=685)	Prevalence%
E.coli	411	60
Klebsiella spp	192	28
Staphylococcus aureus	68	10
Pseudomonas aeruginosa	7	1.02
Enterococci spp	4	0.5
Proteus spp & Acinetobacter spp	4	0.5
Total	685	100

Table 6: Antimicrobial sensitivity pattern of Gram positive organisms

Antibiotics	*Staphylococcus aureus* (n=68)	*Enterococci spp*(n=4)
Penicillin	41.17% (28)	Not tested
Cotrimaxozole	44.11%(30)	Not tested
Erythromycin	57.35%(39)	Not tested
Ciprofloxacin	66.17%(45)	75.00%(3)
Gentamicin	76.47%(52)	Not tested
Cefoxitin	73.52%(50)	Not tested
Vancomycin	100%(68)	100%(04)
Linezolid	100%(68)	100%(04)
Gentamicin (high level)	Not tested	50.00%(2)
Ampicillin	Not tested	75.00%(3)
Nitrofurantoin	40.00%(27)	50.00%(2)

Table 7: Antimicrobial sensitivity pattern of Gram negative organisms

Antibiotics	*E.coli* (n=411)	*Klebsiella spp* (n=192)	*Pseudomonas aeruginosa* (n=07)	*Proteus spp* (n=2)	*Acinetobacter spp* (n=2)
Cotrimaxozole	45.74%188)	41.66%(80)	42.85%(3)	50%(1)	00%(2)
Ciprofloxacin	49.87%(205)	53.64%(103)	57.14%(4)	100%(2)	50%(1)
Norfloxacin	50.36%(207)	57.29%(110)	42.85%(3)	50%(1)	Not tested
Ofloxacin	51.09%(210)	65.10%(125)	57.14%(4)	50%(1)	Not tested
Ceftazidime	53.77%(221)	54.68%(105)	85.71%(6)	100%(2)	50%(1)
Cefuroxime	54.98%(226)	53.64%(103)	57.14%(4)	50%(1)	50%(1)
Amoxyclav	59.12%(243)	58.85%(113)	57.14%(4)	50%(1)	Not tested
Amikacin	63.26%(260)	62.5%(120)	57.14%(4)	100%(2)	50%(1)
Gentamicin	85.60%(352)	87.5%(168)	71.42%(5)	100%(2)	50%(1)
Ampicillin-sulbactam	87.83%(361)	93.75%(180)	Not tested	Not tested	Not tested
Azetronam	72.26%(297)	85.93%(165)	57.14%(4)	100%(2)	50%(1)
Pipercillin-tazobactam	Not tested	Not tested	100%(7)	100%(2)	100%(2)
Nitrofunatoin	53.77%(221)	50%(96)	Not tested	100%(2)	100%(2)
Imipenem	100%(411)	100%(31)	100%(7)	100%(2)	100%(2)

Discussion

The present study provides an overview on the prevalence and anti-biogram of uropathogens which can vary dramatically from time to time and place to place even within the same country. Various factors such as the type of UTI (complicated or uncomplicated), gender, age, and previous history of antibiotic therapy or instrumentation of each patient should also be considered to find out the correct global data on susceptibility. Increasing drug resistance due to empirical treatment in UTI needs regular monitoring of the antibiotic susceptibility of uropathogens in a particular area. To ensure appropriate therapy current knowledge of the pathogens that cause UTI in an area and their susceptibility pattern is mandatory.

In the present study the most common uropathogens isolated was *E.Coli* 411(60%), followed by *Klebsiella* spp 192(28%) *Staphylococcus aureus 68*(10%), *Pseudomonas aeruginosa* 7(1.20%), *Enterococci* spp 4(0.5%), *Proteus* spp and *Acinetobacter* spp 4(0.5%), which was in concordance with the findings carried out by other researchers.[2,8]

The rate of isolation was higher in females(74.59%), thus revealing increased susceptibility of females to UTIs than males(25.40%). The highest isolation was found in the 20-40(40.40%) years age group followed by 61- 80 (37.08%) years age group, thus revealing the increased susceptibility of sexually active females who are more prone to UTIs due to short urethra, proximity to anus and urethral trauma during intercourse and increased vulnerability of the geriatric population to UTIs is presumably due to various age related physiological changes, waning of immune system and other infirmities like diabetes and enlarged prostate, as depicted in other studies.[4,9,10]

The department wise isolation was highest on the surgical department(34.89%) followed by intensive care unit(24.96%), this is may be due to obstructive uropathy, urosurgery,surgical procedures, instrumentation, increased hospital stay and immunosuppression drugs and such similar trends had been reported in other studies.[8,9]

The isolated uropathogens showed wide difference in their susceptibility to tested antimicrobial drugs. The

study showed a very high resistance to betalactam antibiotics and third generartion cephalosporins and to some extent to betalactam/betalactamase inhibitors. Most of the gram negative bacteria showed resistance to Cotrimaxozole, Ciprofloxacin, Ceftazidime, and Amikacin. Most of the uropathogens (50%) showed resistance to nitrofurantoin which is similar to the findings observed by other researchers.[10,11]

The present study also highlights that gram negative bacteria were 100% sensitive to Carbapenems and incidence of Carbapenems resistance is very low in our setup as opposed to increasing trends of Carbapenems resistance shown by other researchers.[9,11] Most of nonfermenters (*Pseudomonas aeruginosa* and *Acinetobacter* spp) showed excellent sensitivity to Carbapenems and Pipercillin-Tazobactam.

In case of gram positive bacteria especially *Staphylococcus aureus* was sensitive to Vancomycin and Linzeloid and showed resistance to Penicillin, Cotrimaxozole, Erythromycin and to some extent to Ciprofloxacin. Similar trends of antibiotic sensitivity pattern was seen in other studies.[12,13] Our study also pointed out that there is an alarming high resistance to Nitrofurantoin (40%) and resistance pattern is on higher side as compared to other researchers.[14,15]

Conclusion

The detection, identification and susceptibility testing of causative species of bacteria are essential for proper treatment and better prognosis of patient in case of UTIs. The present study shows that gram negative bacilli (Enterobacteriaceae) were responsible for majority of urinary tract infections and most common isolated bacteria was *E.coli*. Our study also highlights increasing antibiotic resistance trends in UTI to most commonly used Fluoroquinolones, Cotrimaxozole, Penicillin and alarming resistance was seen with third generation Cephalosporins thus warranting judicious use. Fortunately, in our study almost all gram negative bacteria were sensitive to Carbapenems and also retained useful susceptibility to Aminoglycosides.

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