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Bacteriological and Antibiotic Susceptibility Profile of Uropathogens from a Tertiary Care Hospital

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ABSTRACT

Introduction: The resistance patterns varies significantly in different geographical area. Therefore, routine surveillance is mandatory for constant monitoring of AMR rates among the important pathogens. It is imperative to monitor the changing pattern over time to follow proper therapeutic strategies to control infections due to drug resistant pathogens.

Aim: The aim of this study is to highlight the distribution of various organisms isolated from urine samples, and their susceptibility to different antibiotics collected during 2019 (July to December) in a tertiary care hospital.

Materials And Methods: From 600 urine sample which were received in the microbiology department during the study period, culture was done and organism identification was done using standard conventional biochemical tests and antibiotic susceptibility was carried out according to CLSI guidelines on each of the culture isolates from urine sample.

Conclusion: This study concluded that the majority of the urine samples grew bacteria and few samples grew fungi. *E. coli* was the predominant isolate among them. Gram negative bacteria were more commonly associated with UTI than gram positive organisms. A high level of resistance was seen among the most of the isolates.

Keywords: *urinary tract infection, antibiotic susceptibility testing, antimicrobial resistance,*

1. INTRODUCTION

Urinary tract infection is the second common cause of nosocomial infection worldwide. As described by Farrell et al it accounts for one million hospitalization every year (1,2). Before the culture results are available, antibiotics are given prophylactically because of which there are higher chances for emergence of resistance. There is lack of much studies on organism distribution and resistance pattern of community acquired urinary tract infection. Urinary tract infection is more common in developing countries due to poverty, ignorance and poor hygiene. There is change in microbial distribution and resistance pattern place to place which makes it mandatory to monitor organism distribution and their resistance pattern in a particular area according to Manges et al (3). Therefore, this study was aimed at acquiring knowledge about the organism distribution and their susceptibility patterns which will help the clinicians to choose appropriate antibiotic for empirical treatment (4)

2. MATERIALS AND METHODS

Processing in Laboratory:

Clean catch mid-stream urine sample was collected in a sterile container and sent to the microbiology laboratory. Urine samples were examined macroscopically & processed in the laboratory as soon as possible after collection. In case of delay, the samples were refrigerated.

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: The Urine specimen was inoculated on Blood agar and CLED agar using 0.01 mm calibrated loop for semi-quantitative method. These plates were then incubated overnight in an incubator at 37°C, and observed for growth after 24 hours.

- Counts >105 CFU/ml in midstream urine sample in a patient with no risk factors were considered significant.
- Also >103 CFU/ml in midstream urine sample in a symptomatic patient or in a pregnant female were considered significant

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

Antibiotic Sensitivity Testing: It was carried out on Muller Hinton agar plate by standard Kirby Bauer disk diffusion method as per as CLSI guidelines for commonly used antibiotics (8) The bacterial suspension was made by inoculating 4-5 isolated identical colonies in peptone water and incubated for 2 hours.

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 5 antibiotic discs per plate were placed and inoculated plates were incubated at 37°C. The results were 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), Ampicillin (10µg), Imipenem (10µg), Cefoxitin (30 µg), Pipercillin/Tazobactam (100/10µg), Ceftazidime (30 µg), Amoxyclav (20/10 µg), Cefuroxime (30 µg), Vancomycin (30 µg).

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

Detection of ESBL

According to the CLSI guidelines, isolates showing inhibition zone size of ≤ 22 mm with ceftazidime (30 µg), ≤ 25 mm with ceftriaxone (30 µg), and ≤ 27 mm with cefotaxime (30 µg) were interpreted as positive for ESBL production and shortlisted for confirmation of ESBL production. *E. coli* ATCC 25922 was used as the control strain.

***Confirmation of ESBL production by* E-test**

Confirmation of ESBL was also done by E-test ESBL strips. Double ended strips containing gradient of cefotaxime or ceftazidime at one end and cefotaxime or ceftazidime plus clavulanic acid at the other end were tested. The presence of ESBL was confirmed if the ratio of the MIC of cefotaxime or ceftazidime to the MIC of cefotaxime or ceftazidime plus clavulanic acid was ≥ 8 (CLSI, 2011).

Phenotypic AmpC detection

A modified three-dimensional test was used for the detection of AmpC enzymes in cefoxitin resistant isolates.

3. RESULTS

Out of 600 consecutive urine samples received during the study period, 34 (5.7%) were sterile, 496(82.7%) showed significant growth, 58 (9.7%) showed insignificant growth and 12(2%) were found contaminated. 510(85%) samples were from female patients whereas male compromises of 90 (15%) 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 342(57%) samples were from hospitalized patients while 258(43%) samples were from patients attending outpatient department. Details about different host characteristics are shown

TABLE: 1 TOTAL NUMBER OF SAMPLES (n=600)

Samples	Percentage(%)
Positive samples (496)	82.7
Negative samples (104)	17.3

TABLE: 2 IP/OP DISTRIBUTION

DISTRIBUTION	NUMBER (%)
IP	258(43%)
OP	258(43%)

TABLE: 3 SEX WISE DISTRIBUTION n=496

Sex	Number (%)
Male	74 (12.3%)
Female	342(57%)

TABLE: 4 AGE GROUP WISE DISTRIBUTION n=496

Age group	Numbers	Percentage (%)
< 1 yrs	15	3.02
1-20yrs	51	10.2
21-40yrs	237	47.8
41-60 yrs	49	9.9
60-80yrs	144	29.0
Total	496	100

TABLE: 5 SAMPLE DISTRIBUTION DEPARTMENT WISE n=496

Department	Number of cases	Percentage (%)
Medicine	105	21.1
Surgery	199	40.1
Intensive care unit	113	22.8
Paediatrics	23	4.63
OBGY	56	11.3
Total	685	100

TABLE: 6 DISTRIBUTION OF ORGANSMS ISOLATED FROM URINE SAMPLE

Name of organism	**Numbers (n=496)**	**Prevalence%**
Escherichia coli	315	63.5
Klebsiella spp	52	10.5
Staphylococcus aureus	45	9.07
Pseudomonas aeruginosa	18	3.6
Enterococci spp	4	0.8
Proteus spp	34	6.9
Acinetobacter spp	9	1.8
Citrobacter koserii	4	0.8
Citrobacter freundii	3	0.6
Enterobacter aerogenes	4	0.8

TABLE:7 ANTIBIOTIC SENSITIVITY PATTERN OF GRAM-POSITIVE ORGANISMS

Antibiotics	***Staphylococcus aureus* (n=45)**	***Enterococci spp*(n=4)**
Penicillin	40.3% (18)	Not tested
Cotrimoxazole	42.4%(19)	Not tested
Erythromycin	56.3%(25)	Not tested
Ciprofloxacin	60%(27)	65.00%(3)
Gentamicin	75%(34)	Not tested
Cefoxitin	70%(32)	Not tested
Vancomycin	100%(45)	100%(04)
Linezolid	100%(45)	100%(04)
Gentamicin (high level)	Not tested	40.00%(2)
Ampicillin	Not tested	50.00%(2)
Nitrofurantoin	50.%(22.5)	50.00%(2)

TABLE: 8 ANTIBIOTIC SENSITIVITY PATTERN OF GRAM-NEGATIVE ORGANISMS

Antibiotics	***E.coli* (n=315)**	***Klebsiella spp* (n=52)**	***Pseudomonas aeruginosa* (n=18)**	***Proteus spp* (n=34)**	***Acinetobacter spp* (n=9)**
Cotrimaxozole	54.67%(172)	51.66%(27)	51.85%(9)	40%(14)	100%(9)
Ciprofloxacin	48.87%(154)	52.64%(27)	57.14%(10)	100%(34)	50%(5)
Norfloxacin	50.6%(159)	55.29%(29)	57.85%(10)	40%(14)	Not tested
Ceftazidime	52.7%(166)	50.67%(26)	85.71%(15)	100%(34)	50%(5)

Cefuroxime	52.98%(167)	51.%(27)	47.14%(8)	50%(17)	50%(5)
Amoxyclav	59.12%(186)	57.75%(30)	57.14%(10)	50%(17)	Not tested
Amikacin	62.34%(196)	60.5%(31)	55%(10)	100%(34)	50%(5)
Gentamicin	84%(264)	84.5%(44)	71.42%(13)	100%(34)	50%(5)
Pipercillin-tazobactam	Not tested	Not tested	100%(18)	100%(34)	100%(9)
Nitrofunatoin	53.88%(170)	30%(16)	Not tested	40%(14)	100%(9)
Imipenem	100%(315)	100%(52)	100%(18)	100%(34)	100%(9)
Meropenem	100%(315)	100%(52)	100%(18)	100%(34)	100%(9)

TABLE : 9 ANTIBIOTIC SENSITIVITY PATTERN OF GRAM-NEGATIVE ORGANISMS

Antibiotics	***Citrobacter koseri*** **(n=4)**	***Citrobacter freundii*** **(n=3)**
Cotrimaxozole	43.64% (2)	49.66% (2)
Ciprofloxacin	43.77% (2)	52.64% (2)
Norfloxacin	40.36% (2)	57.09% (2)
Ceftazidime	51.77% (2)	54.18% (2)
Cefuroxime	52.88% (2)	50.64% (2)
Amoxyclav	49.12% (2)	53.85% (2)
Amikacin	60.26% (2)	60.4% (2)
Gentamicin	82.60% (3)	82.2% (2)
Pipercillin-tazobactam	100% (4)	100% (2)
Nitrofunatoin	51.57% (2)	20% (1)
Imipenem	100% (4)	100% (3)
Meropenem	100% (4)	100% (3)

TABLE:10 : Prevalence of ESBL, AmpC β-lactamases,MBL and the coexistence of resistance (ESBL + AmpC β-lactamases) ,(ESBL+MBL) among gram negative bacilli in various clinical isolates

Isolates	**ESBL (%)**	**AmpC (%)**	**MBL (%)**	**ESBL + AmpC (%)**	**ESBL + MBL(%)**
Escherichia coli	158/315(50%)	145/315(46%)	130/315(41.2%)	110/315(34.9%)	78/315(24.7%)
Klebsiella pneumoniae	16/52(11.5%)	12/52(23%)	14/52(26.9%)	8/52(15.3%)	10/52(2%)
Pseudomonas aeruginosa	6/18(33.3%)	4/18(22.2%)	6/18(33.3%)	2/18(11%)	4/18(22.2%)

Proteus mirabilis	12/34(35.2%)	4/34(11.7%)	8/34(23.5%)	4/34(12%)	6/34(18%)
Enterobacter aerogenes	1/4(25%)	1/4(25%)	1/4(25%)	1/4(25%)	1/4(25%)
Citrobacter koseri	1/4(25%)	0/4(0%)	1/4(25%)	0/4(0%)	0/4(0%)
Citrobacter freundii	1/3(33.3%)	0/3(0%)	0/3(0%)	0/3(0%)	0/3(0%)
Acinetobacter spp	1/9(11%)	2/9(22%)	2/9(22%)	1/9(11%)	1/9(11%)

Of the 496 culture positives, isolation of gram-negative bacteria 439(89%) was more than gram positive bacteria 49(10%). *E.Coli* 315 (63.5%) was the most commonly isolated pathogen irrespective of age group and departments followed by *Klebsiella* spp 52 (10.5%), *Staphylococcus aureus* 45 (9.07%), *Pseudomonas aeruginosa* 18 (3.6%), *Enterococci* spp 4(0.8%), *Proteus* spp 34(6.9%) and *Acinetobacter* spp 9(1.8%). The detailed microbiological data of pathogens and their antimicrobial susceptibility causing urinary tract infection are shown in Table 6, 7 & 8.

4. DISCUSSION

The present study gives an insight on the prevalence and anti-biogram of uropathogens which can change depending on various factors. To find the correct data on resistance and susceptibility patterns of uropathogens, various factors like age, gender UTI types of patient should be considered (5). Increasing drug resistance due to irrational treatment of UTI needs regular monitoring of the antibiotic susceptibility of uropathogens. Current knowledge of the pathogens that cause UTI and their susceptibility pattern will aid in the proper therapy of UTI. This study implies that UTI is one of the commonest infections in community, which is similar to the study conducted by **Kibret et al** (6).

More number of patients in this study was females. The isolation rate was higher in females (57%), which implies that females are more prone for urinary infections than male due to their anatomical nature like short urethra which is similar to the study conducted by **Emiru et al** and **Benson et al** (7,8). Highest prevalence of UTI was observed in females with age group of 16-35. This is in agreement with previous studies conducted by **Beyene et al**, **Kibret et al** and **Jombo et al** (9,6,10). The isolation rate was found to be higher in the 20-40(50.40%) years age group followed by 61- 80 (47.08%) years age group, which shows that sexually active females are more prone to UTIs and geriatric population being more prone to UTIs due to factors like presence of comorbid illness like diabetes, hypertension and various age-related changes (11).

Gram negative bacteria were isolated more than gram positive bacteria this is in conformity with other study conducted by **Demille et al** (12). In the present study the most common uropathogens isolated was *E.coli* 315(63.5%), followed by *Klebsiella* spp 52(10.5%) *Staphylococcus aureus 45*(9.07%), *Proteus* spp 34(6.9), *Pseudomonas aeruginosa* 18(3.6%), *Acinetobacter* spp9(2% and *Enterococci* spp 4(0.5%), which was in concordance with the findings carried out by **Saleem et al** and **Bianca et al**(13,14).

Isolation rate was highest on the surgical department (40.1%) followed by intensive care unit (22.8%), which is similar to the study conducted by **Manjunathe et al** (15).

The difference in the distribution of different isolates may be to due difference in geographical location, type of study, climatic conditions and associated comorbid illness.

According to the antibiogram results of this study, *E. coli* was highly susceptible to carbapenem (imipenem and merpenem) and least susceptible to ciprofloxacin (Table1). *Citrobacter koseri* is found to be least susceptible to cotrimoxazole, norfloxacin and ciprofloxacin and *Citrobacter freundi* was least susceptible to nitrofurantoin. *Enterobacter aerogenes* showed resistance to norfloxacin and was sensitive to all other drugs. Proteus was sensitive to amikacin, gentamicin, imipenem and meropenem. *Pseudomonas* was sensitive to most of the antibiotics.

In case of Gram-positive bacteria, *Staphylococcus aureus* was found to be sensitive to Vancomycin and Linezolid and showed resistance to Penicillin and Cotrimoxazole. Similar antibiotic sensitivity pattern was seen in other studies by **Jombo et al** (10). The prevalence of beta lactamases and co-existence of beta lactamases was found to be more in *E. coli* followed by *Klebsiella* spp.

As most of the drugs are available over the counter, people might buy the drugs without prescription. This might also contribute to the emergence of drug resistance. Generally, this study demonstrated that ciprofloxacin, gentamycin, norfloxacin, piperacillin, nitrofurantoin were the current effective drugs for many uropathogens.

5. CONCLUSION

The study concluded that most of the urine samples grew bacteria. *E. coli* being the predominant organism among the other isolates. Gram negative bacteria were commonly associated with the urinary tract infections than gram positive organisms. A high level of resistance was observed in most of the bacterial isolates indicating emergence of resistance due to irrational use of antibiotics. So non-judicious and irrational use of antibiotics should be avoided with proper antibiotic policy. Periodic monitoring of bacteriological profile and their resistance and susceptibility pattern should be done which will help in documenting the changing trend

6. LIMITATIONS

A multicentric study including large number of samples if done, would have increased the importance of this study. Molecular study of the isolates was not done.

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