To Study Antibiotic Sensitivity Pattern of Uropathogens at a Tertiary Care Hospital in Udaipur, Rajasthan

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Abstract: *Objective: The aim of the study to identify different pathogens in urine sample and to study their antibiotic sensitivity pattern at tertiary care hospital in Udaipur, Rajasthan from March 2018 to September201 Material and Method: A total of 500 urine samples were included in the study from patients attended at MBGH, RNT medical college Udaipur with proper sterile precautions. Samples were cultured on HiCrome UTI agar and bacterial isolates obtained were identified using biochemical reactions. Antimicrobial sensitivity testing was performed by the Kirby-Bauer disc diffusion method. Result: A total of 200 uropathogens were isolated from 500 urine samples. Escherichia Coli was the most commonly isolated uropathogen accounting for 47.50% (95/200) of the total isolates followed by Enterococcus accounting for 28% (56/200) of the total isolates. Among Gram negative bacteria Meropenem was found to be most sensitive and among Gram positive cocci Linezolid was found to be most sensitive. Conclusion: Such studies are required for regular monitoring and to obtain reliable information about sensitivity pattern of uropathogens for empirical therapy of patients with UTI. Meropnem should be reserved for empirical treatment in patients with UTI in resistant cases.*

Keywords: Antibiotics Sensitivity Pattern

1. Introduction

Urinary tract infection (UTI) is the commonest bacterial infectious disease in community practice with a high rate of morbidity and financial cost. It has been estimated that 150 million people were infected with UTI per annum worldwide which costing global economy more than 6 billion US dollars.UTI is described as bacteriuria with urinary symptoms.UTI can affect both lower and upper urinary tracts.UTI is more common in females than in males as female urethra structurally found less effective for preventing bacterial entry. It may be due to the proximity of the genital tract and urethra and adherence of urothelial mucosa to the mucopolysaccharide lining. The other main factors which make females more prone to UTI are pregnancy and sexual activity.

The spectrum of bacteria causing complicated UTI is much broader than of those causing uncomplicated UTI. However, the most commonly encountered microorganisms are Gram negative bacteria including *Escherichia coli, Citrobacter spp., Enterobacter aerogenes, Pseudomonas aeruginosa,* and *Proteus vulgaris* whereas *Klebsiella spp., Staphylococcus aureus,* and *Salmonella spp.,* are found rarely.

Increasing multidrug resistance in bacterial uropathogens is an important and emerging public health problem .Increasing drug resistance in UTI needs regular monitoring of antibiotic susceptibility of uropathogens in a particular area.Various factors such as gender, age, previous history of antibiotic therapy of each UTI patient should also be considered to find out the correct data on susceptibility. The distribution of antimicrobial susceptibility data of UTI-causing microorganisms changes from time to time and from place to place. The susceptibility data provided by regional microbiology laboratory helps to choose the empirical choice of antimicrobials to treat UTI. Generally, the antimicrobial treatment is is initiated before the laboratories results which may lead to the frequent misuse of antibiotics.

2. Material and Methods

a) **Study Area:** The study was carried out in the microbiology laboratory of the Department of Microbiology at R.N.T. Medical College, Udaipur (Rajasthan), India. The urine samples were collected from the OPDs (outpatients departments) section of MBGH (Maharana Bhopal Government Hospital) of Udaipur. The duration of the study was 7 Months from March 2018 to September 2018.

b) **Study Population:** The urine samples of 200 patients, comprised of 118 Females and 82 males, who attended the outpatient departments (OPDs) of MBGH hospital were included in this study.

c) **Sample collection:** Clean catch midstream urine was collected from each patient into a 20 mL calibrated sterile screw-capped universal container. As per standard guidelines,the specimens were labeled, transported to the laboratory, and analyzed within 6 hours. All patients were well instructed on how to collect samples aseptically prior to sample collection to avoid contaminations from urethra.

d) **Sample Processing:** A calibrated loop method was used for the isolation of of bacterial pathogens from urinary samples. A sterile 4.0mm platinum wired calibrated loop was used which delivered 0.001mL of urine. A loopful urine sample was plated on MacConkey agar, and blood agar medium .The inoculated plates were incubated at 37°C for 24 h. The number of isolated bacterial colonies was multiplied by 1000 for the estimation of bacterial load/mL of the urine sample.

e) **Identification and Maintenance of pure Bacterial Isolates:** Identification of bacterial isolates was done on the basis of their cultural and biochemical characteristics as per standard guidelines. Gram negative bacteria were identified by the standard biochemical tests and Gram positive microorganisms were identified with the corresponding laboratory tests: catalase, coagulase, and mannitol test for Staphylococcus aureus. The isolates were subcultured periodically. This has been done as per Kass Criteria. { >10*5 cfu/ml indicates significant UTI}

f) **Antibiotic Susceptibility Testing:** Isolates were tested for antimicrobial susceptibility testing by the standard Kirby Bauer's disc diffusion method. Standard inoculums adjusted to 0.5 McFarland was swabbed on Muller Hinton agar and was allowed to soak for 2 to 5 minutes. After that antibiotic discs were placed on the surface of media and pressed gently. Muller Hinton agar plates were then incubated at 37*C for 24h. After 24 h the inhibition zones were measured and interpreted by the recommendations of clinical and laboratory standards. The following standard antibiotic discs were used for the isolates, amikacin, gentamycin, ceftazidime, ceftriaxone, amoxicillin, ampicillin, amoxyclave, ciprofloxacin, norfloxacin, cotrimoxazole, nalidixic acid, nitrofurantoin, tetracycline, cefoxitin, vancomycin, linezolid, piperaillin-tazobactam, meropenem and aztreonam.

3. Results

Total 200 urine samples showed the significant bacterial growth which were comprised of 118 (59%) samples from females and 82 (41%) from males. These results indicated that prevalence of UTI was higher in female patients than in males. A total of 200 bacterial uropathogens comprised of 121 (60.5%) Gram negative and 79 (39.5%) Gram positive were isolated from positive urine samples. *Escherichia coli* was found the dominant bacteria among all isolated uropathogens with the prevalence rate of (47.50%).The second most prevalent isolate was Enterococcus (33%) followed by Staphylococcus aureus (6.5%), Klebsiella

pneumonia (5.5%), Proteus spp. (3%), Pseudomonas aeruginosa (3%), Acinetobacter spp. (1.5%).

Table 1: Frequency of Isolated Bacterial Uropathogens

Bacterial pathogens	Frequency (%)
Escherichia coli	95 (47.5%)
Klebsiella pneumonia	11 (5.5%)
Proteus spp.	6 (3%)
Pseudomonas aeruginosa	6 (3%)
Acinetobacter spp.	3 (1.5%)
Total Gram negative	**121 (60.5%)**
Enterococcus spp.	66 (33%)
Staphylococcus aureus	13 (6.5%)
Total Gram positive	**79 (39.5%)**
Total Gram negative and positive	**200 (100%)**

Antibiotic susceptibility results showed the resistant and susceptible antibiotics for the tested uropathogens. Among Gram negative bacilli Meropenem (99.17%) was found to be most sensitive drug followed by Piperacillin-Tazobactam (80.16%); however, the most resistant drug was Amoxicillin (95.87%) followed by Ceftazidime (85.13%). Meropenem was found to be most sensitive drug (100%) against E.coli and most resistant drug was Amoxicillin (96.85%). 90.91% of K. pneumoniae were resistant against Amoxicillin and Meropenem was found the most susceptible drug with the rate of 90.90%. In case of P. aeruginosa the highest resistant and susceptible antibiotics were Ceftriaxone (83.34%), and Meropenem (100%) respectively. 83.34% of tested Proteus spp. were resistant against Ceftriaxone and 100% sensitive against Meropenem. Acinetobacter spp. Showed 66.67% resistance against Ciprifloxacin and were 100% sensitive to Meropenem. Among Gram Positive cocci Linezolid (83.54%) was found to be most sensitive drug followed by Vancomycin (71.21%); however, the most resistant drug was Ceftazidime (97.47%) followed by Cefoxitin (92.41%). In case of Enterococcus spp. the highest resistant and susceptible antibiotics were Ceftriaxone (96.97%) and Linezolid (84.84%) respectively and in case of S. aureus most resistant and susceptible antibiotics were Ceftriaxone (92.31%) and Vancomycin (100%) respectively.

Isolated Organism	Most Sensitive {%}	Most Resistant {%}
Escherichia coli	Meropenem {100}	Amoxicillin {96.85}
Klebsiella pneumonia	Meropenem {90.9}	Amoxicillin {90.91}
Proteus spp.	Meropenem {100}	Ceftriaxone {83.34}
Pseudomonas aeruginosa	Meropenem {100}	Ceftriaxone {83.34}
Acinetobacter spp.	Meropenem {100}	Ciprofloxacin {66.67}
Total Gram negative bacilli	Meropenem {99.17}	Amoxicillin {95.87}
Enterococcus spp.	Linezolid {84.84}	Ceftriaxone {96.97}
Staphylococcus aureus	Vancomycin {100}	Ceftriaxone {92.31}
Total Gram positive cocci	Linezolid {83.54}	Ceftazidime{97.47}

4. Discussion

This study provides valuable data to compare and monitor the status of antimicrobial resistance among uropathogens to improve efficient empirical treatment. Increasing antimicrobial resistance has been documented globally. Our study showed a high prevalence of UTI in females (59%) than in males (41%).

Resistant and susceptibility rates (%) for isolated uropathogens-

	E. coli (95)		K. pneumonia (11)		Proteus spp. (6)		P.aeruginosa (6)		Acinetobacter Sp (3)		Enterococcus sp (13)		S.aureus	
	%R	%S	%R	%S	%R	%S	%R	%S	%R	%S	%R	%S	%R	%S
AMIKACIN	21.06	78. 94	18.19	81.81	16.17	83.33	16.17	83.33	66.67	33.33	96.97	3.03	15.39	84.61
GENTAMYCIN	41.06	58.94	36.37	63.63	16.17	83.33	33.34	66.66	66.67	33.33	86.37	13.63	38.47	61.53
CEFTAZIDIME	87.37	12.63	72.73	27.27	83.34	16.66	66.67	33.33	100	0	96.97	3.03	100	0
CEFTRIAXONE	86.30	13.70	45.46	54.54	83.34	16.66	83.34	16.66	33.34	66.66	96.97	3.03	92.31	7.69
AMOXICILLIN	96.85	3.15	90.91	9.09	83.34	16.66			100	0	50.0	50.0	84.62	15.38
AMPICILLIN	95.79	4.21							100	0	37.88	62.12	92.31	7.69
CIPROFLOXACIN	72.64	27.36	27.28	72.72	16.17	83.33	33.34	66.66	66.67	33.33	71.22	28.78	84.62	15.38
NORFLOXACIN	76.85	23.15	36.37	63.63	16.17	83.33	66.67	33.33	100	0	84.85	15.15	92.31	7.69
COTRIMOXAZOLE	64.22	35.78	45.46	54.54	66.67	33.33	50.0	50.0	100	0	90.91	9.09	38.47	61.53
NALIDIXIC ACID	85.27	14.73	54.55	45.45					100	0				
NITROFURANTOIN	38.95	61.05	36.37	63.63	100				66.67	33.33	48.49	51.51	7.7	92.20
TETRACYCLINE	57.90	42.10	36.37	63.67	83.34	16.66	83.34	16.66	33.34	66.66	71.22	28.78	15.39	84.61
CEFOXITIN	87.40	12.60	63.64	36.36	83.34	16.66	83.34	16.66	100	0			53.85	46.15
VANCOMYCIN											28.79	71.21	0	100
MEROPENEM	0	100	9.10	90.90	0	100	0	100	0	100				
PIPERACILLIN TAZOBACTAM	21.06	78.94	27.28	72.72	0	100	16.17	83.33	16.17	83.33				
LINEZOLID											15.16	84.84	23.08	76.92

In this study Gram negative bacilli constituted 60.5% of the total bacterial isolates while gram positive cocci constituted 39.5%.

Escherichia coli (47.5%) was found the most prevalent gram negative bacteria in the positive urine samples of UTI. This result is consistent with reports from other studies done in Odisha, Kuwait and Nigeria but differs from the reports in which P.aeruginosa and Klebsiella spp. were recorded as the predominant bacteria in UTI.

The highest incidence of gram negative bacteria, related to Enterobacteriaceae, in causing UTI has many factors which are responsible for their attachment to the uroepithelium.

Meropenem used in this study was found to be the most sensitive drug against all isolated Gram negative bacilli. The sensitivity rate of Meropenem among gram negative bacilli was as follows: *E.coli* (100%), *K. pneumoniae* (90.90%), *Proteus spp.* (100%), *P.aeruginosa* (100%) and *Acinetobacter spp.* (100%). These antibiotic susceptibility results correlate with other studies done in Bangladesh and Pondicherry.

Tested Cephalosporins III generation cephalosporin in this study showed highest resistance among uropathogens as in E.coli; CTZ (87.37%), K.pnuemoniae; CTZ (72.73%), Proteus spp.; CTZ(83.34%), P.aeruginosa; CTZ (66.67%), Enterococcus spp.; CTZ(96.97%) and S.aureus; CFX(92.31%). The possible explanation behind this situation is that the third- generation cephalosporin has been in use for long period and must have been abused and over time organisms have developed resistant mechanisms due to changing their mode of action.

5. Conclusion

This study provides valuable laboratory data to monitor the status of antimicrobial resistance among uropathogens and to improve treatment recommendations.