



National Journal of Medical and Allied Sciences

[ISSN Online: 2319 – 6335, Print: 2393 – 9192|Original article |Open Access]

Website:-www.njmonline.org

BACTERIAL ETIOLOGY AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF URINARY TRACT INFECTION IN SUB-HIMALAYAN REGION OF INDIA - A RETROSPECTIVE STUDY OF CLINICAL ISOLATES

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Abstract

Background: Urinary tract infection (UTI) represents one of the most common diseases encountered in medical practice today. Antibiotics are usually given empirically before the laboratory results of urine culture are available. It accounts for a large proportion of antibacterial consumption and may contribute to the emergence of bacterial resistance. Knowledge of the uropathogens and their antimicrobial susceptibility pattern according to local epidemiology is essential for providing clinically appropriate and cost effective therapy for urinary tract infection.

Objectives: To determine bacterial etiology and antibiotic susceptibility of UTI.

Materials and Method: This retrospective study was conducted between December, 2013 and November, 2014. All urine samples from indoor and outdoor patients of all age groups that were received in our laboratory were studied. The urine samples were inoculated onto CLED (Cystine Lactose Electrolyte Deficient) medium. The urine samples fulfilling the criteria of significant bacteriuria ($\geq 10^5$ colony forming units/ml urine) were included in the study. 2118 (16.09%) of 13,157 urine samples received fulfilled the criteria of significant bacteriuria. Bacteria were identified by standard laboratory methods and antibiotic sensitivity done by Kirby Bauer method.

Results: *Escherichia coli* (E.coli) were the most common bacterial isolates both in outdoor (64.61%) and indoor (58.48%) patients. *E.coli* from both outpatients and inpatients was highly susceptible to nitrofurantoin (96.63% and 88.84%) and amikacin (91.58% and 92.27%). Overall susceptibility of urinary isolates to nitrofurantoin from outpatients and inpatients was 89.28% and 83.90% respectively. The corresponding figures for ampicillin were 26.43% and 21.14%, for norfloxacin were 29.24% and 19.27%, and for cotrimoxazole were 35.32% and 26.26%.

Conclusion: *E. coli* continues to be the most common cause of UTI. Bacteria causing UTI are less sensitive to commonly used antibiotics for its treatment. Nitrofurantoin appears to be highly effective oral antibiotic for UTI. It should be used judiciously to prevent the increase in resistant strains. Culture and sensitivity of the isolates from urine samples should be done as a routine before advocating the therapy and local antibiogram should guide the choice of antibiotic therapy.

Keywords: Urinary tract infection (UTI), antimicrobial resistance, multidrug resistance, enterobacteriaceae

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Introduction:

Urinary tract infection (UTI) represents one of the most common diseases encountered in medical practice today with an estimated 150 million UTIs per annum worldwide.^[1] It is defined as the disease caused by invasion of the urinary tract by

microorganisms. Antibiotics are usually given empirically before the laboratory results of urine culture are available. To be successful, the empirical treatment provided must be guided by clinical evidences as well as the safety profile and cost-effectiveness of the drug, and adhere to

antimicrobial stewardship. [2] UTI accounts for a large proportion of antibacterial consumption and may contribute to the emergence of bacterial resistance. [3] Although UTI occurs both in men and women, clinical studies suggest higher prevalence in women. An estimated 50% women suffer one attack of UTI at some point in a life time and 20%-40% suffer from recurrent episodes. [4, 5] *E.coli* is the most common causative agent, and other bacteria include the species of *Klebsiella*, *Proteus*, *Pseudomonas*, *Enterobacter*, *Citrobacter*, *Staphylococcus* and *Enterococcus*. The members of enterobacteriaceae account for approximately 75% of isolates of UTI samples. [6]

Though introduction of antimicrobials have contributed significantly to the management of UTI, however rapid development of resistance among microorganisms is a major problem in the current antibiotic therapy. [7] Knowledge of the uropathogens and their antimicrobial susceptibility pattern according to local epidemiology is essential for providing clinically appropriate and cost effective therapy for UTI. [8] Pattern of antibiotic sensitivity may vary even over short periods and from place to place, and periodic evaluation of antibiotic sensitivity is needed to update this information. [9] Since most UTIs are treated empirically the selection of antimicrobial agent should be determined not only by the most likely pathogen but also by its expected susceptibility pattern. In this context the present retrospective study was designed to determine the local microbial etiology of UTI in outdoor and indoor patients and to determine the microbial susceptibility to the most commonly used antimicrobials. To the best of our knowledge, this is the first study to evaluate the susceptibility patterns of bacterial strains isolated from patients of UTI in Shimla, HP. It provides a valuable laboratory data to enable clinicians to devise and endorse a rational antibiotic policy to reduce the incidence of UTI, and allows comparison

of the situation in this hilly state with other parts of the country.

Material and method:

The present retrospective study was conducted in the department of Microbiology of Indira Gandhi Medical College, Shimla, after clearance from institutional ethics committee. The clinical laboratory records of all urinary samples processed from December 2013 to November 2014 were sought and those with positive culture reports were studied and analysed. Majority of the samples were midstream urine specimens, and others included catheterised urine samples and suprapubic aspirates. Only one specimen per patient was included. The urine samples were cultured on CLED (Cystine Lactose Electrolyte Deficient) medium with calibrated loop technique, a semiquantitative method, delivering 0.001mL of urine and colony counts of ≥ 100 (10^5 CFU/ml) were considered significant. Any numbers of bacteria were considered significant for suprapubic aspirates. The isolates were identified by standard microbiological methods. [10] Antimicrobial sensitivity was done by Kirby Bauer disc diffusion method on Mueller Hinton Agar plates using a panel of antibiotics as per CLSI guidelines. [11] The antibiotic discs and their disc concentrations per disc (μg) included: ampicillin (10), trimethoprim-sulfamethoxazole (25), norfloxacin (10), ciprofloxacin (5), nitrofurantoin (300), amikacin (10), cefexime (5), ceftazidime (30), ceftazidime/clavulanic acid (30/10), cefoxitin (30), piperacillin (100), piperacillin/tazobactam (100/10), imipenem (10) and linezolid(30). Methicillin resistance in *staphylococcus* spp, and extended spectrum β lactamase (ESBL) production in *E.coli* and *Klebsiella* spp. was done as per CLSI guidelines. [11] An isolate was considered as MDR (multidrug resistant) if resistance to three or more antimicrobials belonging to different classes or

groups of antimicrobials was found. [12] The source of Mueller Hinton Agar (MHA) and antibiotic discs was Hi Media, India. The data was analysed by Epi info version 3.3.2 and P value <0.05 was taken as significant.

Results:

Out of 13,157 samples received in the department (age range 2months to 103 years), 2,118(16.09%) were bacterial culture positive and 55 (0.42%) were positive for Candida species. Mixed growth was reported in 2,911 (22.11%) samples and 8074 (61.36%) samples were sterile. Out of 2,118 culture positive patients, 1670(78.89%) were outdoor patients and 448(21.15%) were from inpatient department. Among the total patients studied 1184 (55.90%) were females and 934 (44.09%) were males.

E.coli was the most common isolate in both outdoor and indoor patients, but was significantly higher ($P= 0.01$) in urines from outpatients than inpatients. The proportion of *Klebsiella* species and enterococci was significantly higher ($P<0.05$) in inpatients (12.05% and 12.27% respectively) than in outpatients (8.02% and 8.80% respectively). The uropathogens isolated during the study period are depicted in table 1.

The percentage of susceptible isolates varied for different organisms, and among outpatients and inpatients (Table 2). Of all the bacteria from outpatients 89.28% (1466/1642) were sensitive to nitrofurantoin and 88.84% (1219/1372) were sensitive to amikacin, and the percentage susceptibles for ampicillin, cotrimoxazole, norfloxacin and cefexime were 26.42% (202/764), 35.32% (538/1523), 29.24% (475/1624) and 47.56% (352/740) respectively. Bacteria from inpatients also showed high sensitivity for nitrofurantoin (83.91%, 370/441) and amikacin (85.82%, 321/374) and low sensitivity for ampicillin, cotrimoxazole and norfloxacin and

cefexime (Table 2). The susceptibility of *E.coli* to ampicillin, cotrimoxazole, norfloxacin, nitrofurantoin, amikacin, cefexime, piperacillin-tazobactam and imipenem was 21.48%, 49.29%, 27.55%, 91.89%, 91.72%, 45.92%, 91.4% and 98.2% respectively.

Resistance was significantly more frequent in *E.coli* from inpatients than in *E.coli* from outpatients for all the antibiotics tested ($P<0.05$) except for amikacin where the resistance level was almost similar. We observed similar level of resistance in *Klebsiella* spp for norfloxacin and cotrimoxazole both from outpatients and inpatients. *Staphylococcus aureus* showed high sensitivity to nitrofurantoin and amikacin both in outpatients and inpatients (Table 3). We tested for methicillin resistance among all isolates of *staphylococcus* spp as a part of our routine laboratory procedure. A significant difference ($P=0.0003$) was seen in MRSA detection in inpatients (48.38%, 21/44) and outpatients (21.60%, 39/183). ESBL production in *E.coli* and *Klebsiella* spp together was 30.80%, significantly more frequent ($P=0.00002$) in inpatients than in outpatients (42.34% and 27.12%). Overall ESBL production was significantly ($P=0.02$) higher in *Klebsiella* species (39.16%, 47/120) than in *E.coli* (29.09%, 272/935). Multi drug resistant isolates of *E.coli* were seen in 19.61% (263/1341). No isolate of *Staphylococcus* spp and *Enterococcus* spp was found resistant to linezolid. *Pseudomonas* spp showed high sensitivity to amikacin (81.57%), piperacillin (78%), piperacillin-tazobactam (89.2%), ceftazidime (84.06%) and imipenem(94%), and only 48.83% and 44.14% isolates were sensitive to nitrofurantoin and norfloxacin respectively. It showed a relatively lesser resistance towards norfloxacin than *E.coli* and *Klebsiella*. (Table 3)

Table 1: Bacterial etiology of urinary tract infection from outpatients and inpatients

Antimicrobials	E.coli		Klebsiella spp		S.aureus		CoNS		Enterococcus spp		Pseudomonas spp	
	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD
Ampicillin	23.09	14.18	19.04	11.53	22.72	7.14	44.44	00	45.83	47.27	0	0
Cotrimoxazole	36.95	25.19	33.83	33.33	35.13	32.35	33.33	37.50	ND	ND	3.12	0
Norfloracin	29.87	18.21	33.84	33.33	25.85	11.42	27.77	12.50	17.12	10.90	47.05	33.33
Nitrofurantoin	92.63	88.84	79.10	62.96	95.94	97.14	86.11	100	90.47	87.27	47.05	55.55
Amikacin	91.58	92.27	75.83	65.21	89.47	90.32	82.75	75.00	73.33	81.81	83.33	75.00
Cefexime	50.24	30.35	38.23	37.93	ND	ND	ND	ND	ND	ND	16.66	14.28

Table 2: Percentage of susceptible isolates (%) to common antimicrobials

Antimicrobial	Outpatients	Inpatients
Ampicillin	26.43	21.14
Cotrimoxazole	35.32	26.26
Norfloracin	29.24	19.27
Nitrofurantoin	89.28	83.91
Amikacin	88.84	85.82
Cefixime	47.56	30.59

Table 3: Sensitivity (% susceptible) of the most frequently isolated bacteria

Bacteria	Outpatients		Inpatients	
	Number	%	Number	%
<i>E.coli</i>	1079	64.61	262	58.48
<i>Staphylococcus aureus</i>	148	8.86	35	7.86
Coagulase negative staphylococcus	36	2.15	8	1.78
<i>Klebsiella spp</i>	134	8.02	54	12.05
<i>Enterococcus spp</i>	147	8.80	55	12.27
<i>Enterobacter spp</i>	28	1.67	10	2.23
<i>Pseudomonas spp</i>	34	2.03	9	2.00
<i>Proteus spp</i>	20	1.19	7	1.56
<i>Morganella morganii</i>	2	0.11	0	0
<i>Citrobacter spp</i>	18	1.07	2	0.44
Non-fermenter group of organisms	20	1.19	6	1.33
<i>Providencia spp</i>	4	0.23	0	0
Total	1670	100	448	100

Discussion:

The changing trends in etiopathogenesis of UTI and emerging resistance to the antibiotics, is a matter of concern worldwide. Despite the advances in and wide spread availability of antimicrobials, UTI still remains the most common infection in hospitalised and outdoor patients. The indiscriminate and inadequate usage of antimicrobials has further increased the emergence of resistant strains. [13] The present study is a retrospective study using the results of our routine diagnostic and susceptibility analyses. This study reflects that the pathogens causing UTI in outdoor and indoor setup show different percentages of prevalence (Table 1). The culture positivity in our study is (16.09%) comparable to that done in Jaipur, India. [14] Among the culture positive outdoor patients females (57.36%) exceeded in number than males (42.63%).

This finding reveals increased susceptibility of females to UTI than males in the community. This observation is in concordance with other previous studies.^[15,16,17] Females are more prone to develop UTI, probably due to their characteristic anatomical and physiological changes like short urethra, its proximity to the anus, urethral trauma during sexual activity, dilatation of urethra and stasis during pregnancy.^[13] The gender difference was negligible in the indoor patients. UTI may be more common in females in outdoor set up but hospitalised males may be having some co morbid conditions such as renal stones, obstruction of urinary outflow, surgical procedures, instrumentation, strictures, immunosuppressive drugs etc which act as risk factors for development of UTI in admitted males.^[18] This study demonstrates that *E. coli* remains the leading uropathogen being responsible for 63.30% cases of UTI in our area, which is in accordance with findings of other studies around the globe.^[15,19,20] Besides *E. coli*, our study shows *S.aureus*, (8.86%, 7.82%), *Enterococcus* spp (8.80%, 12.27%) and *Klebsiella* spp (8.02%, 12.27%) as other common organisms in outdoor and indoor settings respectively. Our findings are in concordance with studies done in India and other parts of the world.^[14,21] In our study, bacteria of Enterobacteriaceae family accounted for 76.48% of all the isolates, followed by gram positive cocci (20.20%) and non-fermenter gram negative bacteria (3.20%) (Table1). Our study reveals 29.09% of the *E. coli* isolates and 39.16% of *Klebsiella* species to be ESBL producers which are in accordance with a study done by Bours PH et al.^[22] Wide variation is reported in various studies in ESBL producing bacteria over the globe which could be due to the different pattern of antibiotic usage in different geographic regions.^[14, 23, 24] It is emphasized that we must use appropriate tests for detection of ESBL producing bacteria to avoid indiscriminate use of third generation of cephalosporins.

Multidrug resistant *E. coli* were observed in 19.61% isolates. The prevalence of MDR in urinary isolates of *E.coli* reported in literature varies in different studies and may reflect the prevalence of MDR strains in the community and hospital.^[25, 26] The antimicrobial resistance among uropathogens is one of the barricades that might interfere with an effective treatment. The antimicrobial susceptibility pattern of different urinary isolates is depicted in table 3. High degree of resistance observed in our study towards ampicillin, cotrimoxazole and norfloxacin among all urinary isolates from outpatients as well as inpatients raise concern over the use of these agents. Our findings are in consistent with data reported in some recent studies.^[15, 16, 17] Indiscriminate and irrational use, and easy availability and over the counter sale of antibiotics without proper prescription and dosing schedule, and their wide usage for a variety of other indications might have led to resistant strains. A notable observation was that majority of the isolates showed a higher sensitivity towards nitrofurantoin and amikacin. Nitrofurantoin was found to be an effective oral antibiotic in majority of the patients. Our findings are similar to other Indian studies which have also demonstrated nitrofurantoin as an appropriate agent for first-line treatment particularly for community acquired UTIs.^[27, 28]

The high sensitivity to nitrofurantoin among common oral antibiotics for treatment of UTI may be influenced by its narrow spectrum of activity, limited indications, limited tissue distribution, and limited contact with bacteria outside the urinary tract.^[29] However we observed a higher degree of resistance to nitrofurantoin (11.85%) similar to that reported by Grude N et al.^[30] It can be explained by the fact that nitrofurantoin is one of the most commonly prescribed antibiotics for treatment of UTI in our set up. We are also aware that not all urines from patients with UTI are sent to laboratory and they are usually treated without bacteriological

testing. Considering the fact that nitrofurantoin has no role in the treatment of other infections, it can be administered orally and is highly concentrated in urine; it may therefore be the most appropriate agent for empirical use in uncomplicated UTI. Amikacin, an aminoglycoside also shows high sensitivity (88.84%/85.82%; outdoor/indoor) among most urinary isolates. Aminoglycosides being injectable are used restrictively for treatment of uncomplicated UTI and hence have shown better sensitivity rates. For gram positive cocci nitrofurantoin and amikacin were effective whereas the common antibiotics like ampicillin and norfloxacin showed higher degree of resistance. MRSA and ESBL production was higher in hospitalised patients than in outdoor patients. These isolates are usually multi-resistant and there may be selection for the multi-resistant phenotypes in hospitals where there is a higher consumption of antibacterial drugs. *Pseudomonas* spp showed a high degree of resistance to ampicillin, cotrimoxazole and third generation cephalosporins, and lower susceptibility to nitrofurantoin and fluoroquinolones. Amikacin, ceftazidime, piperacillin and piperacillin-tazobactam are the antibiotics recommended for UTI with *Pseudomonas* spp. The rising resistance to nitrofurantoin among uropathogens is alarming and warrants its cautious use. It may be wise to let some antibacterial drugs rest for some years until sensitivity to these drugs has reached an acceptable level.

Conclusion:

In view of the emerging drug resistance amongst bacteria therapy should only be advocated, as far as possible, after culture and sensitivity testing. Bacterial etiology and antibacterial susceptibility may change over time with in a population. Medical microbiology laboratories should monitor and report any such changes in bacterial etiology and susceptibility, and every institution should have its

own antibiotic policy based on local susceptibility pattern so that treatment of UTI can be optimised in order to reduce increasing bacterial resistance.

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Conflicts of Interest: None Funding: None

Citation: Mokta KK, Mokta JK, Verma S, Singh D, Kanga A. Bacterial Etiology and Antibiotic Susceptibility Pattern of Urinary Tract Infection in Sub-Himalayan Region of India - A Retrospective Study of Clinical Isolates. *National Journal of Medical and Allied Sciences* 2015; 4(1):38-45