Etiology and antimicrobial susceptibility pattern of bacterial pathogens from urinary tract infection

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ABSTRACT

Urinary tract infection (UTI) is the most common infection in both community and hospital patients. In majority of the cases, empirical antimicrobial treatment is practiced before the laboratory results of urine culture. Thus, antibiotic resistance may increase in urinary bacterial pathogens due to improper use of drugs. This study was designed to find out the etiological agents of UTI and their prevalence, and to determine the antimicrobial susceptibility pattern of the bacterial pathogens isolated from urine culture. This study was conducted in Kathmandu Model Hospital, Kathmandu, Nepal from April to October, 2009. Midstream Urine samples from 1323 patients suspected of UTI were analyzed by microscopy, and conventional semi-quantitative culture technique for the significant growth. Antimicrobial susceptibility test was performed for the isolates by Modified Kirby-Bauer disk diffusion method. Data were analyzed using SPSS software window version 16. The overall prevalence of UTI was found to be 18.89%. The most frequent causative organisms isolated were *Escherichia coli* (82.30%), *Enterococcus faecalis* (5.60%), *Citrobacter freundii* (3.60%), *Enterobacter aerogenes* (2.40%), Coagulase Negative Staphylococci (2.40%), *Pseudomonas aeruginosa* (1.20%), *Proteus mirabilis* (0.8%), *Klebsiella pneumoniae* (0.4%), and *Staphylococcus aureus* (0.4%). Nitrofurantoin and Amoxycillin were found to be the most effective antibiotic against gram negative and gram positive isolates respectively. *E. coli* was found to be the most common etiological agent of UTI and Nitrofurantoin was the most effective drug among the isolates.

Keywords: UTI, etiology, midstream urine, antibiotic.

INTRODUCTION

Urinary tract infection (UTI) is one of the most common infections described among outpatient settings and hospital patients[1, 2] with an estimated annual global incidence of at least 250 million cases.[3] It has higher rate of occurrence among females than males which may be due to anatomical, hormonal, and other physiological differences between them.[4, 5] It is estimated that about one-half of all women will have at least one episode of UTI during their lifetime[6] and there is increase in occurrence of UTI during pregnancy by about 7%.[7] Among outpatients, UTI is caused by *E. coli, K. pneumoniae, P. mirabilis, S. saprophyticus,* or *E. faecali* while in hospitalized patients it is caused by *E. coli, Ps. aeruginosa, Proteus* spp*., Enterobacter* spp*., Serratia* spp*. or E. faecalis.*[8, 9]

UTI encompasses both asymptomatic bacteriuria and symptomatic infection with microbial invasion and inflammation of urinary tract.[10] While up to 90% of the patients with UTI complain of urinary tract symptoms, one-third or more of the patients with these symptoms do not have bacteriuria.[11] The most common symptoms for which most patients seek treatment are dysuria and frequency.[12] These symptoms together raise the probability of UTI more than 90%, effectively ruling in the diagnosis by history alone.[13]

In almost all cases empirical antimicrobial treatment of UTI begins before the laboratory results of urine culture are available. So, the antimicrobial resistance may increase in uropathogens as a result of improper use of drugs which is of global concern.[14-17]

For this reason, the knowledge of etiology of UTI and their antimicrobial resistance patterns may help in choosing appropriate antimicrobials for the empirical treatment of the disease. Thus present study was undertaken to find out etiology of UTI and its prevalence, and to determine the antimicrobial susceptibility pattern of commonly used antibiotics.

MATERIALS AND METHODS

Between April to October 2009, 1323 patients (401 males and 922 females) visiting Kathmandu Model Hospital, Kathmandu with symptoms related to urinary tract infection (UTI) were screened for the symptomatic bacteriuria. Patients who had no symptoms suggestive of UTI at the time of observation were excluded from the

Table-1: Etiology and Isolation rate of uropathogens

	Patients		
	Male (%).	Female (%)	**Total**
E. coli	53 (21.2)	155 (62)	**208**
E. faecalis	11 (4.4)	3 (1.2)	**14**
Pseudomonas aeruginosa	2 (0.8)	1 (0.4)	**3**
CoNS	2 (0.8)	4 (1.6)	**6**
Others	6 (2.4)	13 (5.2)	**19**
Total	74	176	250

study. Those patients who refused to provide necessary information were not included in the study.

Necessary information was collected using interview technique from each respondent. A structured questionnaire was used to assess the study subjects' self reported information regarding urinary symptoms.

All study subjects were advised to aseptically collect the mid-stream urine samples in wide-mouthed sterile containers. The samples were processed within 1 hour of collection. For direct microscopy 50µl of well-mixed uncentrifuged urine was taken on a slide and a cover slip placed on it. It was viewed under a high-power objective. The presence of one pus cell/7 high power field was considered significant pyuria.[18]

For urine culture, the sample was inoculated each on MacConkey Agar and Blood Agar using a standard loop following semi-quantitative method. The plates were observed after 24 hours of aerobic incubation at 37°C. A single organism obtained in counts of >100,000 cfu/ml was further identified by standard biochemical tests.[19]

Modified Kirby Bauer disc diffusion technique was used for antimicrobial susceptibility testing. A bacterial suspension matched to 0.5 McFarland was lawn cultured on Mueller Hinton Agar. The appropriate antimicrobial discs were then placed firmly onto the surface of plates using sterile forceps. The plates were then incubated at 37°C for 18-24 hours. Interpretation of results was done using the standard zone sizes.[20]

Data analysis was performed using the SPSS windows version 16 software. A value of $p<0.05$ was considered statistically significant.

Following Operational Definitions[18] were put to use in the present study:

- Microscopy findings of more than 10 WBC per high power field were considered significant.
- Significant bacteriuria was defined as culture of a single bacterial species from the urine sample at a concentration of more than 100,000 cfu/ml.

RESULTS

The prevalence of UTI was found to be 18.89% (5.59% in males and 13.30% in females). A total of 250 uropathogens were isolated, out of which 229 (91.60%) isolates were Gram negative rods and 21 (8.4%) isolates were Gram positive cocci (Table-1).

The common urinary symptoms presented were burning sensation during micturition (62.40%), frequency (33.70%), urgency (21.0%), painful voiding (15.20%), and nocturnal incontinence (4.10%).

Escherichia coli was the most common uropathogen isolated, and responsible for UTI in 82.30% patients. *Enterococcus faecalis* was isolated in 5.60%, C*itrobacter freundii* in 3.60%, *Enterobacter aerogenes* in 2.40%, Coagulase Negative Staphylococci (CoNS) in 2.40%, *Pseudomonas aeruginosa* in 1.20%, *Proteus mirabilis* in 0.8%, *Klebsiella pneumonia* in 0.4% and *Staphylococcus aureus* 0.4% of patients (Table-2).

The pattern of antibiotic susceptibility of *E. coli* was: Ampicillin 32.70%, Cefotaxime 75.96%, Ciprofloxacin

Table-2: Percent susceptible for antimicrobial agents against Gram negative uropathogens

Organism (N, % of UTI)	Antimicrobials							
	AM	CE	CF	CFX	CO	NF	NX	OF
	AM	CE	CF	CFX	CO	NF	NX	OF
E. coli (208, 83.2%) % susceptible	32.7	75.96	64.42	75	52.4	99.03	63.46	62.98
C. freundii (9, 3.6%) % susceptible	44.44	66.66	77.77	33.33	77.77	0.00	77.77	77.77
K. pneumoniae (1, 0.4%)% susceptible	0.00	0.00	0.00	0.00	0.00	100	0.00	0.00
E. aerogenes (6, 2.4%) % susceptible	0.00	100	100	83.33	83.33	66.66	83.33	83.33
P. mirabilis (2, 0.8%) % susceptible	100	100	100	100	100	50	100	100

Table-3: Percent susceptible for antimicrobial agents against P. aeruginosa

Organism (N, % of UTI)	Antimicrobials							
	AK	CE	CF	CO	CA	CI	G	NX
P. aeruginosa (3, 1.2) % susceptible	100	50	66.67	66.67	33.33	66.67	100	50

64.42%, Cefexime 75.0%, Cotrimoxazole 52.40% and Nitrofurantoin 99.03%. *E. faecalis* had susceptibility of: Amoxicillin 85.71%; Ciprofloxacin 42.80%; Cotrimoxazole 42.85%; Norfloxaxin 35.71%; Cephalexim 22.20%; Erythromycin 60.0% and Cloxacillin 14.80%. *Pseudomonas aeruginosa* isolated had the similar pattern of antimicrobial susceptibility results as Amikacin 100%; Ciprofloxacin 66.67%; Cotrimoxazole 66.67%; Ceftazidime 33.33%; Ceftriaxone 66.67% and Gentamicin 100 % (Table-3).

E. coli was more frequent in women (62%) than in males (21.20%). The isolation rate of *E. faecalis* was 4.40% in males and 1.20% in females.

The most effective antibiotic for the *E. coli* isolates observed was Nitrofurantoin (99.03%) followed by Cefexime (75.96%), Ciprofloxacin (64.42%), Norfloxacin (63.46%), and Ofloxacin (62.98%). Similarly, *Klebsiella* isolates were 100% susceptible to Nitrofurantoin. Amikacin and Gentamycin were observed highly effective (100%) against *Pseudomonas aeruginosa.* Amoxicillin (85.71%) followed by Erythromycin (60%) were most effective against *Enterococcus faecalis* isolates. Cefexime, Ciprofloxacin, Cephalexim and Erythromycin were highly effective (100%) for Coagulase Negative Staphylococci (CoNS). Staphyloccous aureus isolate was the most susceptible (100%) to Cefexime, Norfloxacin and Cloxacillin (Table-4).

DISCUSSION

The prevalence of UTI was found to be higher in females than in males. Among females the prevalence of UTI (61.93%) was higher in the middle age patients (20-49 years). Similar results with higher prevalence of UTI in females were observed in other studies carried out under similar clinical settings.[21, 22]

The drier environment surrounding the male urethra, may prevent the optimal growth of bacteria compared with the female urethra.[23] The antibacterial activity of prostatic secretions in men is also a factor that reduces the risk of UTI in men. In men, there is also a much longer distance between the anus and the urethral meatus.[4] Genital microflora plays an important role in women's defenses against invasions, so any alterations (e.g. due to antibiotherapy and its adverse effects upon the genital ecology) can enhance genital colonization with uropathogens and risk of subsequent UTI.[4] Another common reason for high prevalence among female may be due to poor hygienic conditions. The fecal flora *E. coli* which contaminates perineum may have caused bacteriuria in females.[24-26] Females may have lower urinary cytokine concentrations and therefore decreased urinary leukocyte numbers which may be the reason behind higher prevalence of bacteriuria in them.[27]

The predominant number of pathogens isolated in our study were Gram negative bacilli rather than Gram positive pathogens. Bacteriological studies usually reveal the involvement of Gram negative enteric organisms that commonly cause UTI, such as *E. coli*, *Klebsiella* species and *Proteus* species.[28] Similarly, in a study, the most predominant pathogens isolated from UTI were Gram negative bacilli.[29] The higher prevalence of gram negative enteric organisms in UTI cases may be due to the better chances of these organisms getting access to urinary tract from the intestine where they inhabit as normal flora.

Gram positive cocci play a lesser role in causing UTI. In our study too gram positive cocci were isolated only from 8.40% of patients. There was found the involvement of *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in UTI which suggests that patients' defenses against these organisms may have been reduced. They may have developed UTI as a hospital acquired infection.

Table-4: Percent susceptible for antimicrobial agents against Gram positive

Organism (N, % of UTI)	Antimicrobials							
	AM	CE	CF	CO	NX	CP	E	CX
E. faecalis (14, 5.6%) % susceptible	85.71	57.14	42.8	42.85	35.71	22.2	60	14.8
CoNS (6, 2.4 %)% susceptible	50	100	100	50	50	100	100	80
S. aureus(1, 0.4%)%susceptible	0.00	100	NA	0.00	100	-	0.00	100

AK= Amikacin, AM= Amoxycillin, CA= Ceftazidime, CE= Cefotaxime, CF= Ciprofloxacin, CFX= Cefexime, CI= Ceftriaxone, CO= Cotrimoxazole, CP= Cephalexim, CX= Cloxacillin, E= Erythromycin, G= Gentamicin, NF= Nitrofurantoin, NX= Norfloxacin, OF= Ofloxacin, N= Number of isolates

Gram negative bacilli were found to be highly sensitive to Nitrofurantion, Cefotaxime and Cefexime. Nitrofurantoin was the most effective drug observed against *E. coli* followed by Cefexime and cefotaxime. The greater effectiveness of Nitrofurantoin in the treatment or UTI have been increasingly reported.[30] Amikacin was the most effective drug against *Pseudomonas aeruginosa* with 100% susceptibility. Similarly gram positive cocci were found to be more susceptible towards Amoxycillin and Erythromycin. The only isolate of *Staphylococcus aureus* was 100% susceptible to Cefotaxime, Norfloxacin and Cloxacillin which is incongruence with other study where all isolates of *S. aureus* were found to be susceptible to Imipenem, Ceftraixone and Cefotaxime.[21]

Improper use of antibiotics for the treatment of disease prior availability of laboratory culture results has increased the chances of empirical treatment failure. So, it is necessary to know the etiology and determine the antimicrobial susceptibility of bacterial isolates to prevent treatment failure and emergence of drug resistant strains.

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