Frequency and susceptibility profile of pathogens causing urinary tract infections at a tertiary care hospital in western Nepal

Das R N, Chandrashekhar T S, Joshi H S, Gurung M, Shrestha N, Shivananda P G

ABSTRACT

Introduction: Urinary tract infection (UTI) is the most common nosocomial infection among hospitalised patients. Area-specific monitoring studies aimed to gain knowledge about the type of pathogens responsible for UTIs and their resistance patterns may help the clinician to choose the correct empirical treatment. Recent reports have shown increasing resistance to commonly-used antibiotics. We aimed to study the antibiotic resistance pattern of the urinary pathogens isolated from hospitalised patients.

Methods: Three urine samples were collected by the mid-stream "clean catch" method from 1,680 clinically-suspected cases of urinary tract infections from inpatients of various clinical departments during one year. The samples were tested microbiologically by standard procedures. Antibiotic susceptibility of the isolated pathogens was tested for commonly-used antibiotics by Kirby-Bauer technique according to NCCLS guidelines.

Results: Significant bacteriuria was present in 71.7 percent of the samples, 17 percent were sterile, 4.8 percent showed insignificant bacteriuria, and 6.5 percent non-pathogenic bacteriuria. The most common pathogens isolated were *Escherichia coli* (59.4 percent), *Klebsiella* spp (15.7 percent) and *Enterococcus faecalis* (8.1 percent). The mean susceptibility was high for amikacin (87.2 percent), ciprofloxacin (74.8 percent), ceftazidime (71.5 percent) and gentamicin (70.4 percent) but low for nitrofurantoin (35 percent), cephalexin (49.7 percent) and ampicillin (50.5 percent). *Escherichia coli* was found to be most susceptible to amikacin (98 percent) followed by gentamicin (87.9 percent), ceftazidime (80.8 percent), norfloxacin (78.4 percent) and cotrimoxazole (77.9 percent).

Conclusion: A high isolation rate of pathogens from urine samples of clinically-suspected UTI shows a good correlation between clinical findings and microbiological methods. The antibiotics commonly used in UTIs are less effective. Since the present study was a cross-sectional study, regular monitoring is required to establish reliable information about resistance pattern of urinary pathogens for optimal empirical therapy of patients with nosocomial UTIs.

Keywords: antibiotics, bacteriuria, nosocomial infection, urinary tract infection

Singapore Med J 2006; 47(4):281-285

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(1). With advancing age, the incidence of UTI increases in men due to prostate enlargement and neurogenic bladder(2). Recurrent infections are common and can lead to irreversible damage of the kidneys, resulting in renal hypertension and renal failure in severe cases(3). In the community, women are more prone to develop UTI. About 20% of women experience a single episode of UTI during their lifetime, and 3% of women have more than one episode of UTI per year(4). Pregnancy also makes them more susceptible to infection(5). Catheter-associated UTI is a trenchant problem, with about 5% of catheterised patients developing bacteriuria(6). It is universally accepted that UTI can only be ascertained on the basis of microscopy and microbial culture. The dipstick/dip-slide method used in many centres serves only as a screening method but culture is needed for the final diagnosis(7).

UTI is the most common cause of nosocomial infection among hospitalised patients(1). In almost all cases, there is a need to start treatment before the final microbiological results are available.

Department of Medicine
Manipal Teaching Hospital
Manipal College of Medical Sciences
PO Box No 341
Pokhara
Nepal

Das R N, MD, MRCP
Assistant Professor

Department of Microbiology
Manipal Teaching Hospital

Gurung M, BSc
Postgraduate Student

Shrestha N, BMLT
Postgraduate Student

Shivananda P G, MD, PhD
Professor and Head

Department of Community Medicine
Manipal College of Medical Sciences
PO Box 155
Deep Heights
Pokhara
Nepal

Chandrashekhar T S, MD
Lecturer

Joshi H S, MD, DCH
Lecturer

Correspondence to:
Dr T S Chandrashekhar
Tel: (977) 61 521 387
Fax: (977) 61 522 160
Email:chandrashekharats@yahoo.com

Area-specific monitoring studies aimed to gain knowledge about the type of pathogens responsible for UTIs and their resistance patterns may help the clinician to choose the correct empirical treatment. Studies from the eastern part of Nepal, India and Bangladesh have reported an increased resistance of the urinary pathogens to commonly-used antibiotics[8-10]. Such information was not available for a tertiary care hospital in western Nepal. Hence this study was undertaken to find out the frequency and antibiotic susceptibility pattern of the urinary pathogens isolated from the urine samples of suspected nosocomial urinary tract infections at a tertiary care hospital in western Nepal.

METHODS

The present study was conducted at the Manipal Teaching Hospital of Manipal College of Medical Sciences, a 750-bed tertiary care hospital located at Pokhara city in western Nepal. All the urine samples received from the inpatients of various clinical departments of Manipal Teaching Hospital from August 1, 2003 to July 30, 2004 were selected for the study. If there were more than two episodes of UTI for the same patient, either due to prolonged hospitalisation or repeated hospitalisations, each episode was considered as a separate case of UTI. The patients who had symptoms and/or signs suggestive of UTI were suspected as having UTI.

Three urine samples were collected by standard mid-stream "clean catch" method from all the patients with suspected urinary tract infection. Three urine samples were also collected from catheterised patients, if the catheter was not inserted for not longer than 72 hours. The samples collected were examined microscopically for pus cells and casts. The samples were also processed using standard microbiological procedures. All the urine samples were processed on the blood agar and MacConkey's medium by standard loop method and incubated at 37°C overnight. The plates were observed for bacterial growth.

Culture results were interpreted as being significant and insignificant, according to the standard criteria. A growth of $\geq 10^5$ colony forming units/mL was considered as significant bacteriuria[11]. Patients with significant bacteriuria and symptomatic patients with lower colony counts were also considered as having UTI. The organism was identified by routine methods from the samples showing significant bacteriuria[12]. Cultures with more than three colonies were discarded, as contaminants and their antibiotic susceptibility were not tested. Antibiotic susceptibility tests and interpretations were carried out for bacterial isolates by the Kirby-Bauer technique[13]. Antibiotic susceptibility was tested by disc diffusion method for all the first and the second line antibiotics, according to the hospital antibiotic policy, irrespective of the isolated organism showing susceptibility/resistance to the first line antibiotics.

The first line antibiotics tested were: ampicillin, oxacillin, cephalexin, nitrofurantoin, cotrimoxazole, gentamicin, amikacin, nalidixic acid and norfloxacin, and the second line were: ciprofloxacin, ceftazidime and piperacillin (Hi-Media Pvt. Laboratories, Bombay, India). Appropriate quality control strains were used to validate the results of the antimicrobial discs. The following were the quality control strains used: *Pseudomonas* spp-NCTC 10662, *Staphylococcus aureus*-NCTC 6571, *Escherichia coli (E. coli)*-NCTC 10418, *Enterococcus*-NCTC 12697[14]. Guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) were followed for the testing and interpreting the antimicrobial disks. Data was entered into Microsoft Excel and analysed. The results were expressed as proportions and mean susceptibility was calculated for first line and second line antibiotics for each organism isolated by culture.

RESULTS

The age-sex distribution of the samples, according to the culture positive cases, is shown in Table I. Out of the 1,291 culture positive urine samples, 109 (8.4%) were from children aged ten years or less, and 277 (21.4%) were from those aged 60 years and above. In all age groups, except those aged 0-10 years, females were more frequently affected than males. 389 (23.2%) out of 1,680 urine samples examined did not yield any organism. There were 64 urine samples obtained from catheterised patients and 1,141 urine samples collected from non-catheterised samples. Out of the 1,680 samples processed, 1,205 (71.7%) showed significant bacteriuria, 286 (17%) were sterile, 80 (4.8%) showed insignificant bacteriuria, and 109 (6.5%) non-pathogenic bacteriuria. *E. coli* and *Klebsiella* spp were commonest organisms isolated from both catheterised and non-catheterised samples (Table II).

The organisms grown on the culture of all the 1,205 samples with significant bacteriuria were as follows: *E. coli* 716 (59.4%) *Klebsiella* spp 189 (15.7%), *Enterococcus faecalis* 98 (8.1%), *Proteus mirabilis* 89 (7.4%), *Staphylococcus aureus* 41 (3.4%), *Pseudomonas aeruginosa* 35 (2.9%), *Staphylococcus*

Table I. Age and sex distribution of the culture-positive urine samples.

Age (in years)	Gender		Total
	Male	Female	
0-10	68	41	109 (8.4%)
11-20	108	122	230 (17.8%)
21-40	81	189	270 (20.9%)
41-60	95	310	405 (31.4%)
Above 60	135	142	277 (21.4%)
Total	487 (37.7%)	804 (62.3%)	1,291 (100%)

Table II. Bacteria isolated from urine samples of catheterised and non-catheterised patients.

Organism	Catheterised	Non-catheterised	Total
E. coli	31 (48.4%)	685 (60%)	716 (59.4%)
Klebsiella spp	20 (31.2%)	169 (14.8%)	189 (15.7%)
Enterococcus faecalis	1 (1.5%)	34 (2.9%)	98 (8.1%)
Proteus mirabilis	9 (14%)	80 (7%)	89 (7.4%)
Staphylococcus aureus	1(1.5%)	97 (8.5%)	41 (3.4%)
Pseudomonas aeruginosa	2 (3.1%)	39 (3.4%)	35 (2.9%)
Staphylococcus saprophyticus	–	26 (2.2%)	26 (2.1%)
Citrobacter freundii	–	11 (0.9%)	11 (0.9%)
Total	64	1,141	1,205

saprophyticus 26 (2.1%) and *Citrobacter freundii* 11 (0.9%) (Table II).

The antibiogram of isolated pathogens is shown in Table III. The highest susceptibility was for amikacin (87.2%), ciprofloxacin (74.8), ceftazidime (71.5%), and gentamicin (70.4%) for all the pathogens isolated; and the least was for nitrofurantoin (35%), cotrimoxazole (45.8), cephalexin (49.7%) and ampicillin (50.5%). *E. coli* was most sensitive to amikacin (98%), followed by gentamicin (87.9%), ceftazidime (80.8%), norfloxacin (78.4%) and cotrimoxazole (77.9%).

Klebsiella spp was most susceptible to ceftazidime (96.8%), norfloxacin (76.2%) and ciprofloxacin (95.7%). *Pseudomonas aeruginosa* was most sensitive to piperacillin (100%) amikacin (100%) ciprofloxacin (98.2%) and ceftazidime (97.3%). *Staphylococcus aureus* was most sensitive to amikacin (100%) and ciprofloxacin (85.7%) but least susceptibility was observed for cotrimoxazole (12.5%) and ampicillin (13.8%). Since *Staphylococcus saprophyticus* was not a common cause of UTI, antibiotic susceptibility was not tested.

DISCUSSION

The age and sex distribution of the patients diagnosed with UTI among the hospitalised patients followed the natural epidemiological pattern of UTI. There was a higher number of young male children. Similarly, there was a greater predominance of young and middle-aged females, whereas in the older age group (≥60 years), equal proportions of

Table III. Bacteria isolated from all the urine samples and their susceptibility pattern to common antibiotics.

Organism isolated	No (%) n=1205	Antibiotic susceptibility (%)											
		Amp	Cpx	Nfx	Gm	Amk	Oxc	Nfn	NA	Cmz	Cfz	Cfx	Ppc
E. coli	716 (59.4)	72.6	65.9	78.4	87.9	98.0	–	68.4	69.9	77.9	80.8	55.0	–
Klebsiella spp.	189 (15.6)	33.8	11.1	76.2	60.8	61.3	–	2.1	51.8	28.5	96.8	95.7	–
P. mirabilis	89 (7.3)	57.1	50.0	62.9	83.3	87.6	–	13.4	68.5	56.1	–	85.7	–
P. aeruginosa	41 (3.4)	50.0	75.0	–	88.8	100	–	–	–	–	97.3	98.2	100
S. aureus	98 (8.1)	13.8	73.6	–	64.5	100	96.2	66.3	–	12.5	–	85.7	–
S. faecalis	35 (2.9)	58.2	11.2	8.2	14.2	68.6	–	–	9.1	60.0	11.2	10.5	–
S. saprophyticus	26 (2.1)	–	–	–	–	–	–	–	–	–	–	–	–
Citrobacter	11 (0.9)	68.1	61.3	–	93.5	94.8	–	24.8	80.3	39.9	–	92.8	–
Mean susceptibility		50.5	49.7	56.4	70.4	87.2	96.2	35.0	55.9	45.8	71.5	74.8	100

Antibiotic susceptibility test was not done for *Staphylococcus saprophyticus*.

First line antibiotics: Amp: Ampicillin; Cpx: Cephalexin; Gm: Gentamicin; Amk:Amikacin; Oxc: Oxacillin; Nfx: Norfloxacin; NA: Nalidixic Acid; Nfn: Nitrofurantoin; Cmz: Cotrimoxazole

Second line antibiotics: Cfx: Ciprofloxacin; Ppc: Piperacillin; Cfz: Ceftazidime

Mean susceptibility = Sum of susceptibility of individual organisms/total no of organisms.

males and females had UTI. Diagnosis of UTI is a good example of the need for close cooperation between the clinician and the microbiologist. The presence of significant bacteriuria in 71.6% of the samples shows a good correlation between clinical and bacteriological diagnosis, and also indicates the significance of microbiological culture to clinch the diagnosis of UTI.

In the present study, insignificant bacteriuria and sterile cultures were observed in 4.8% and 17%, respectively. Prior antibiotic treatment before submitting the urine sample, and clinical conditions such as non-gonococcal urethritis or other clinical conditions that mimic UTI could be the factors responsible for such results. Furthermore, 6.5% of the urine samples showed presence of non-pathogenic bacteria. This indicates the need for educating the patients about the method collection of "clean catch" urine specimens.

Enterobacteriacae have several factors responsible for their attachment to the uroepithelium. These gram-negative aerobic bacteria colonise the urogenital mucosa with adhesin, pili, fimbriae and P1-blood group phenotype receptor[15]. In the present study, the *Enterobacteriacae* group, namely, *E. coli* (59.4%), *Klebsiella* spp. (15.7%), *Enterococcus faecalis* (8.1%), and *Proteus mirabilis* (7.4%), were the most common pathogens isolated, followed by gram-positive cocci, namely, *Staphylococcus aureus* (3.4%) and *Staphylococcus saprophyticus* (1.4%). The isolation rates of urinary pathogens are consistent with reports of the recently-published studies.[9, 16, 17]

Isolation rate for *Staphylococcus aureus*, which is a rare cause of UTI, was 3.4%. However, it is interesting to note that only one case occurred out of 64 catheterised patients, whereas the rate was 8.1% for non-catheterised patients. In our study sample, there were 321 patients who were diagnosed to have either renal calculus, hydronephrosis who could have had prior catheterisation, or may even be suffering from bacteraemic kidney infection. Hence, there could have been high isolation of *Staphylococcus aureus* from the cases of nosocomial UTI in our study.

E. coli, which was the principal pathogen isolated, showed high susceptibility to common antibiotics ampicillin (72.6%), cotrimoxazole (77.9%) and norfloxacin (78.4%). This is contrary to the reports of studies from other countries which have reported an increasing resistance to ampicillin[9,17-19]. A study from eastern Nepal reported a high prevalence of resistance to nalidixic acid, ampicillin and norfloxacin[8]. Another study from Bangladesh reported an increased resistance of uropathogens to ciprofloxacin[10]. Ciprofloxacin and norfloxacin, which are used equally for UTI caused by *Klebsiella* spp has demonstrated increased resistance to both antibiotics and also sometimes cross-resistance exists. But in the present study, *Klebsiella* spp showed high susceptibility to both ciprofloxacin and norfloxacin. This is contrary to the results of the above-mentioned studies which reported an increased resistance of the uropathogens to both ciprofloxacin and norfloxacin.

Pseudomonas aeruginosa, which is a common cause of hospital-acquired UTI, was less sensitive to the common antibiotics but highly sensitive to amikacin, piperacillin, ciprofloxacin and gentamicin. Similar results are reported from other countries[17,20]. *Pseudomonas* was susceptible to the second line drugs and most of these are associated with high resistance to the first line antibiotics used, namely, ampicillin and ciprofloxacin. This may be due to widespread clinical use of common antibiotics in the hospital and cross-resistance existing among various classes of antibiotics. Even though *Pseudomonas aeruginosa* is known to be resistant to ampicillin, and first and second generation cephalosporins, we tested the susceptibility of *Pseudomonas* to these antibiotics to determine its susceptibility pattern in our setting.

The highest resistance was shown by *Klebsiella* spp which was the second most common organism isolated from the UTIs. *Klebsiella* spp was resistant to commonly-used antibiotics, except ceftazidime (96.8%) which was the second line antibiotic according to hospital antibiotic policy. A similar pattern was observed for *Proteus mirabilis*, which was susceptible to amikacin, ciprofloxacin and gentamicin (87.6%, 85.7 % and 83.3%, respectively). The gram-positive organisms isolated from urinary samples also showed an increased resistance against common antibiotics except for the *Staphylococcus aureus* which showed high susceptibility to oxacillin (96.2%).

In the present study, among the cephalosporins, cephalexin showed low mean susceptibility (49.7%) but ceftazidime showed high mean susceptibility (71.5%). *Enterococcus* showed low susceptibility to cephalexin (11.2%) and ceftazidime (11.2%). A report from the SENTRY Antimicrobial Surveillance Program, USA has shown similar results[17]. Various *Citrobacter* spp isolated might have had inducible beta-lactamases which was not tested in the present study. The results of the present study showed that the mean susceptibility of the uropathogens was low for cotrimoxazole which often is one of the first line drugs in many countries. This low susceptibility could be due to widespread use of this antibiotic in the community. It is possible that low susceptibility is prevalent in the nosocomial UTI as well as community-acquired UTI. Some of the hospitalised patients may be having community-acquired UTI. In our study, we could not distinguish between community-acquired UTI and nosocomial UTI. This was the main limitation of the study.

In conclusion, a high isolation rate of pathogens from urine samples of clinically-suspected UTI shows a good correlation between clinical findings and microbiological methods. Gram-negative organisms were the commonest organisms isolated, among which *E. coli* was the principal urinary pathogen. Data presented in this study indicate that antibiotics commonly used for the treatment of nosocomial UTIs are less effective. Since this was a cross-sectional study, further regular monitoring is required to establish reliable information about resistance pattern of urinary pathogens for optimal empirical therapy of patients with nosocomial UTI.

ACKNOWLEDGEMENTS

The authors thank all staff and faculty of Department of Microbiology, Manipal Teaching Hospital, for their cooperation and help. The authors are also thankful to Dr Chiranjy Mukhopadhyay for his valuable suggestions for revisions of the final manuscript, and to Dr P Ravi Shankar for revising and proof-reading the manuscript.

REFERENCES

1. Ronald AR, Pattulo MS. The natural history of urinary infection in adults. Med Clin North Am 1991; 75:299-312.
2. Liperky BA. Urinary tract infection in men: epidemiology, pathophysiology, diagnosis and treatment. Ann Intern Med 1989; 111:138-50.
3. New CH. Urinary tract infection. Am J Med 1992 (suppl); 4A: 63-70.
4. Gebre-Selassie S. Asymptomatic bacteriuria in pregnancy: epidemiological, clinical and microbiological approach. Ethiop Med J 1998; 36:185-92.
5. Pastore LM, Savitz DA, Thorp JM Jr, et al. Predictors of symptomatic urinary tract infection after 20 weeks' gestation. J Perinatol 1999; 19:488-93.
6. Tambyah PA, Maki DG. Catheter associated urinary tract infection is rarely symptomatic; a prospective study of 1497 catheterized patients. Arch Int Med 2000; 160:678-82.
7. Van Nostrand JD, Junkins AD, Bartholdi RK. Poor predictive ability of urinalysis and microscopic examination to detect urinary tract infection. Am J Clin Pathol 2000; 113:709-13.
8. Srinivasa H, Parija SC, Bhattacharya S, Sehgal R. Incidence of ciprofloxacin resistance in urinary isolates Eastern Nepal. J Comm Dis 1999; 31:45-7.
9. Navaneeth BV, Belwadi S, Suganthi N. Urinary pathogens' resistance to common antibiotics: a retrospective analysis. Trop Doct 2002; 32:20-2.
10. Iqbal J, Rahman M, Kabir MS, Rahman M. Increasing ciprofloxacin resistance among prevalent urinary tract bacterial isolates in Bangladesh. Jpn J Med Sci Biol 1997; 50:241-50.
11. Stamm WE. Measurement of pyuria and its relation with bacteriuria. Am J Med 1983; 75:53-8.
12. Cruickshank R. Duguid JP, Marnian BP, Swain RH. Tests for identification of bacteria. In: Medical Microbiology, Vol II, 12th ELBS ed. London: Churchill Livingstone, 1975: 170-89.
13. Bauer AW, Kirby WMM, Sherris JC, Turch M: Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol 1966; 45:493-9.
14. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. Eighth informational supplement 2000. Villanova, PA: National Committee for Clinical Laboratory Standards (NCCLS). Approved standard M2 A7.
15. Lomberg H, Hanson LA, Jacobsson B, et al. Correlation of P blood group, vesicoureteral reflux, and bacterial attachment in patients with recurrent pyelonephritis. N Engl J Med 1983; 308:1189-92.
16. Abu Shaqra Q. Occurrence and antibiotic sensitivity of Enterobacteriaceae isolated from a group of Jordanian patients with community acquired urinary tract infections. Cytobios 2000; 101:15-21.
17. Jones RN, Kugler KC, Pfaller MA, Winokur PL. Characteristics of pathogens causing urinary tract infections in hospitals in North America: results from the SENTRY Antimicrobial Surveillance Program, 1997. Diagn Microbiol Infect Dis 1999; 35:55-63.
18. Rossi A, Tokumoto M, Galas M, Soloaga R, Corso A. [Monitoring antibiotic resistance in Argentina. The WHONET program, 1995-1996] Rev Panam Salud Publica 1999; 6:234-41. Spanish.
19. Allen UD, MacDonald N, Fuite L, Chan F, Stephens D. Risk factors for resistance to "first-line" antimicrobials among urinary tract isolates of Escherichia coli in children. CMAJ 1999; 160:436-40.
20. Kahlmeter G; ECO.SENS. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO.SENS Project. J Antimicrob Chemother 2003; 51:69-76.