*Journal of Institute of Science and Technology 2018, **23**:39-42*
ISSN: 2469-9062 (print), 2467-9240 (e)
DOI: https://doi.org/10.3126/jist.v23i1.22159

Research Article

MULTI-DRUG RESISTANT UROPATHOGENS ISOLATED FROM MID STREAM URINE SAMPLES

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***(Received:** August 14, 2018; **Revised**: December 1, 2018; **Accepted:** December 4, 2018)*

ABSTRACT

A frequent and consistent evaluation of the prevalence, etiologic agents and predisposing factors of urinary tract infection (UTI) is necessary in developing country like Nepal to reduce its severe consequences. A regular and routine monitoring of multidrug resistant (MDR) clinical isolates is essential in laboratory practice. Five hundred and ninety one mid stream urine samples were collected from Bharosa Hospital of Kathmandu and a semi-quantitative method was used to determine the frequency of MDR uropathogens in the urine samples. Isolation, identification and antimicrobial susceptibility of isolated bacteria were done by standard microbiological procedure. From the total samples, 161 samples were found to be positive for UTI (27.24 %). Among these bacterial isolates, 57.14 % were found to be MDR. Nitrofurantoin, amikacin and ceftriaxone were found to be the most effective antibiotics against uropathogens.

Keywords: Urinary tract infection, Multidrug resistance, Uropathogens, Antimicrobial Susceptibility Test, Midstream urine

INTRODUCTION

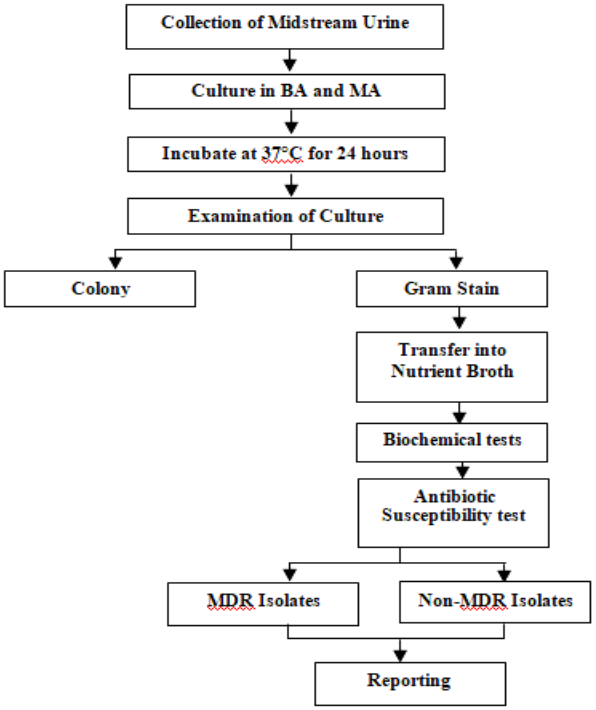
Urinary tract infection (UTI) is a spectrum of disease caused by microbial invasion of genitourinary tract that extends from the renal cortex of kidney to the urethral meatus. UTIs are among the most frequent infectious diseases affecting humans and represent an important public health problem with a substantial economic burden. In the USA, every year more than 7 million people with UTI come to visit physician and 15 % of them use prescribed antibiotic (Bonkat *et al.* 2017). It was reported that UTI positive rate in Nepalese patient attending general hospital range from 23.1 % to 37.4 % (Rai *et al.* 2001). Nepal has about 61.4% people illiterate, who do not have concept of hygiene and thus are vulnerable to infections. In many parts of Nepal, the facilities for urine culture and antimicrobial susceptibility testing are not available leading to incorrect diagnosis of UTI. Antibiotics are usually given empirically before the laboratory results of urine culture are available. To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory (Chakraborty 2001).

The problem of antimicrobial resistance may be due to the fact that antibiotics are easily assessable without medical authorization or supervision in developing countries (Pokharel *et al.* 2006). This situation has led to inappropriate usage of antibiotics with patients taking the drugs for insufficient length of time or at sub optimal dosages, which may result in antimicrobial resistance. The antimicrobial resistance is a serious emerging problem throughout the world. Multiple drug resistant (MDR) bacterial isolates have been frequently reported from different parts of the world as an emergence of treatment problem. Antibiotic susceptibility profile and reporting of drug resistant strain would provide information for the appropriate antibiotic therapy. So, this study was intended to address hospital acquired bacterial isolates causing UTI. The result of this study will also help clinicians to facilitate the empirical treatment of patients with symptoms of UTIs. Moreover, the data would also help relevant authorities to formulate antibiotic prescription policies.

MATERIALS AND METHODS

A prospective cross-sectional study was carried out in diagnostic laboratory of microbiology in a hospital from October 2015 to March 2016. During this period, a total of 591 urine samples from patients suspected of UTI were collected and were processed according to the standard laboratory methods (Cheesbrough 2006). Urine culture was performed in MacConkey agar and blood agar to detect the presence of significant bacteriuria. Diagnosis of UTI was proceeded when at least 10^5 organisms were present per mL of urine samples. Identification was done using morphological, cultural and biochemical tests as described by Vandepitte *et al.* (2003) and details of the processes are shown in the flowchart below (scheme 1).

Antibiotic susceptibility testing (AST) of bacterial isolates was done by Kirby-Bauer disc diffusion method following CLSI guidelines. The antibiotic discs used for this study were amikacin (30 µg), cefalexin (30µg), cefixime (5 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), gentamicin (10 µg), levofloxacin (10 µg), nitrofurantoin (300 µg) and ofloxacin (5 µg).



Scheme 1. Flowchart for processing of the clinical specimens

RESULTS

Out of 591 mid stream urine samples, 161 (27.24 %) samples showed significant growth whereas majority of the samples 430 (72.76 %) showed non-significant growth (Table 1). In this study, the age of the patient ranged between 18 months to 93 years. Among the 161 significant positive cultures, highest percent 38 (23.75 %) of significant growth culture was obtained from patients of age group between 21-30 years that was followed by age group 41-50 years 22 (13.66 %) as depicted in Table 2. A total of 9 species of bacteria belonging to seven different genera were isolated from significant bacteriuria urine samples, which are shown in Table 3. Among the isolates *Escherichia coli* (65.84 %) was found to be the most predominant organism followed by *Klebsiella pneumoniae* (12.42 %).

Table 1. Number of midstream urine samples and growth of bacterial isolates

Growth Pattern	Urine Samples	
	No.	%
Significant growth	161	27.24
Non-significant growth	430	72.76
Total	**591**	**100**

E.coli was found to be most sensitive to nitrofurantoin 71 (66.98 %), followed by ceftriaxone 69 (65.09 %) and resistant to cotrimoxazole 48 (45.28 %) and cefalexin 27 (25.47 %). *Klebsiella* sps was found to be sensitive to amikacin 15 (75 %) followed by ceftriaxone and resistant to ofloxacin 10 (50 %) (Table 4). Among the isolated organisms, 92 (57.14 %) were found to be MDR. *E.coli* was the most prevalent isolate that is 58 (63.04 %) (Table 5).

Table 2. Sex-wise and age-wise distribution of the isolates

Age Group (years)	Male		Female		Total No.	%
	No.	%	No.	%		
0-10	1	16.67	5	83.33	6	3.7
11-20	2	22.22	7	77.78	9	5.6
21-30	2	5.26	36	94.74	38	23.6
31-40	2	10.0	18	90.00	20	12.4
41-50	6	27.27	16	72.73	22	13.7
51-60	10	47.62	11	52.40	21	13.1
61-70	2	15.38	11	84.62	13	8.1
71-80	5	27.78	13	72.22	18	11.2
81-90	3	30.0	7	70.00	10	6.2
91-100	2	50.0	2	50.00	4	2.4
Total	**35**		**126**		**161**	**100**

(χ^2 value = 19.41153, p-value = 0.021916)

Table 3. Distribution pattern of different uropathogens isolated from urine samples

Bacteria	Frequency	%
E.coli	106	65.8
Klebsiella pneumoniae	14	8.7
Klebsiella oxytoca	6	3.7
Enterococcus	16	10
Enterobacter	13	8.1
Pseudomonas aeruginosa	3	1.9
Staphylococcus aureus	1	0.6
Staphylococcus saprophyticus	1	0.6
Proteus vulgaris	1	0.6
Total	**161**	**100**

Table 4. Antibiotic susceptibility pattern of *E. coli* (n=106) and *Klebsiella* spp (n=20)

Antibiotics	*E. coli*		*Klebsiella spp*	
	Susceptible No.	%	Susceptible No.	%
Amikacin	68	64	15	75
Cefalexin	16	15	3	15
Cefixime	49	46	9	45
Ceftriaxone	69	65	12	60
Ciprofloxacin	40	38	12	60
Cotrimoxazole	48	45	10	50
Gentamicin	62	59	7	35
Levofloxacin	50	47	5	25
Nitrofurantoin	71	67	10	50
Ofloxacin	30	28	4	20

Table 5. MDR patterns of isolated organisms

Organisms	Total isolates	MDR No.	%
E coli	106	58	63.04
Klebsiella pneumoniae	14	13	14.13
Klebsiella oxytoca	6	3	3.30
Enterococcus	16	8	8.70
Enterobacter	13	7	7.53
Pseudomonas aeruginosa	6	3	3.30
Total	**161**	**92**	**100**

DISCUSSION

In this study, prevalence of UTI was found to be 27.24 % which is relatively lower than reported in the study of Mishra *et al.* (2005) and Shrestha *et al.* (2005). This might be due to patients taking antibiotics prior to urine culture or infection due to slow growing organisms or fastidious organisms. Out of 161 UTI positive cases, infection was found to be higher in female compared to male. In addition, high infection rate was found in age group of 21-30 year as compared to other age groups ($p < 0.05$). Out of 126 female patients, showing significant growth of urine culture, 36 (94.74 %) female patients were from this age group. In young women, sexual activity is the cause of 75-90 % of bladder infections, with the risk of infection related to the frequency of sex (Nicole 2001). A urinary tract infection is of concern in pregnancy due to increased risk of kidney infections (Dielubanza *et al* 2011). Sexual activity is an important factor in the pathogenesis of UTI in female.

In this study, 92 (57.5 %) were MDR; it means they were resistant to two or more than two different structural classes of antibiotics. Present finding is in consistence with the study done by Baral (2008), Pokharel (2006), Poudyal (2011) and Shrestha *et al.* (2005) which showed 47.94 %, 39.69 %, 60.40 %, 64.6 % and 55.87 % MDR isolates, respectively. In a study by Bomjan (2005), the highest proportion (68.33 %) of MDR among urinary isolates was reported. The consistent and high-level susceptibility of *E. coli* to nitrofurantoin may be influenced by nitrofurantoin's narrow spectrum of activity, limited indication (treatment of acute cystitis), narrow tissue distribution (low or undetectable serum concentrations) and limited contact with bacteria outside the urinary tract (Jones *et al*. 1999). In a study done by Kurutepe *et al.* (2005), 100 % of MDR *E coli* isolates were resistant to ampicillin whereas 80.6 %, 74.1 % and 33.3 % of them were resistant to ciprofloxacin, gentamicin, nitrofurantoin, respectively. High resistance rate of MDR *E coli* isolates to norfloxacin found in the present study is a great concern. A gradual decrease in the susceptibility of *E. coli* to fluoroquinolones (approximately 1 % per annum) has also been reported by the US arm of the SENTRY surveillance program, with no change in susceptibility to nitrofurantoin (Jones *et al.* 1999, Mathai *et al.* 2001). Increasing fluoroquinolones resistance among urinary *E. coli* has also been documented in studies conducted outside the US (Goettsch *et al.* 2000). The increasing prevalence of MDR Enterobacteriaceae limits available treatment options for infections caused by these organisms. Due to the lack of new antibiotics, there is a valid rationale for testing older antibiotics that retain some activity against MDR bacteria.

CONCLUSION

This study showed that *E.coli* 106 (65.83 %) was the major organism in UTI followed by *Klebsiella pneumoniae* (12.42 %). Majority (57.14 %) of the isolates included in this study was found resistant to two or more antibiotics (MDR). *E. coli* was found to be the most predominant MDR isolates followed by *Klebsiella pneumoniae*. Moreover, this study concluded that all *E. coli* isolates and most of other Gram-negative bacteria isolates were sensitive to amikacin, ceftriaxone and nitrofurantoin so they can be used as a drug of choice for the treatment of UTI. Similarly, Gram positive bacteria were found the most sensitive to nitrofurantoin. Hence, nitrofurantoin can be the best choice of antibiotics for UTI.

ACKNOWLEDGEMENT

Authors are deeply indebted to Mr. Kamal Pandey for providing of laboratory facilities.

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