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Study of Culture and Sensitivity Pattern In Urinary Tract Infections in A Tertiary Care Center in Nepal

Binod Chapagain[1], Parshal Bhandari[2], *Binod Aryal[1]

[1]Tribhuvan University Teaching Hospital(TUTH), Kathmandu, Nepal
[2]Medical Officer, Nepal Medical College and Teaching Hospital, Kathmandu, Nepal
*Email: aryalbinod17@gmail.com
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Abstract: The main purpose of this study was to find out the causative agents of urinary tract infections (UTI) and their culture and antibiotic sensitivity in patients visiting Tribhuvan University and Teaching Hospital (TUTH). A retrospective study conducted among 155 patients, aged from 25-50 years with culture-positive UTI, who visited TUTH from 1st April 2017 to 30th September 2017. A culture of midstream urine was done to find out causative agents and their antibiotic sensitivity performed. Data were evaluated using Microsoft Excel 2016. Female were more affected than males. Escherichia coli (E. coli) was the most common microbes causing UTI in 53% patients. Most of the isolates on culture were Multi-Drug Resistant (MDR) strains to comprise 52%. Of the total gram-negative organisms, 33.9% were Extended Spectrum β-lactamase (ESBL) producers, and 3.57% were Metallo β-lactamase (MBL) producers. 29.41% of *Staphylococcus* were resistant to methicillin. *E.coli* is the most common organism causing UTI among adults. Multidrug-resistant has appeared alarming with resistant to most of the first line antibiotics.
Keywords: Urinary tract infection (UTI); Antibiotics; β-lactamase; Resistant

INTRODUCTION

Microbial invasion of any tissue from renal cortex to urethral meatus is considered as Urinary Tract Infection (UTI) (Obiogbolu et al., 2009). About 10% of population experience UTI in their lifetime is one of the most common infectious disease (Farajnia et al.,2009). UTI is more common in females than males and rarely occurs in men without any functional or anatomical abnormalities along urinary tract (Stamm et al., 2001). Sex (distance between anus and urethral meatus,shorter in female), age (low concentration of lactobacillus in elder female), personal hygiene, pregnancy, use of birth control pills, immune-suppressive conditions, diabetes, instrumentation of urinary tract are some of the risk factors predisposing to UTI (Flores-Mireless et al., 2015). UTI can be classified as symptomatic or asymptomatic, complicated or uncomplicated and upper or lower urinary tract infections (Behzadi et al., 2010).

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This study might be useful for the health care providers in Nepal to understand current scenario of UTI and rational use of antibiotics for its treatment.

MATERIALS AND METHODS

The undertaken study conducted in the Department of Microbiology, TUTH, Kathmandu, Nepal from 1st April 2017 to 30th September 2017. Total 155 patients, aged between 25 -50 years, with culture-positive UTI, were involved. The causative organisms isolated in culture and their antibiotic sensitivity pattern was retrospectively studied.

Mid-stream urine samples collected from the patients with urinary control and catheter samples collected from those without urinary control. Urine microscopy along with culture and antibiotic sensitivity performed in the lab of TUTH. Kirby-Bauer disk diffusion method was used for Antibiotic Susceptibility Test. Nitrofurantoin, amikacin, norfloxacin, levofloxacin, cotrimoxazole, cefixime, ceftriaxone, ceftazidime, cephalexin, gentamycin, amoxicillin and cloxacillin were tested as first-line antibiotics whereas Imipenem, polymyxin-B, colistin-sulfate, chloramphenicol, tigecycline, piperacillin, piperacillin-tazobactam, cefoperazone, cefoperazone-sulbactam, ampicillin-sulbactam, cefepime, vancomycin, teicoplanin, clindamycin, tobramycin, and amoxicillin-clavulanic acid taken as second-line antibiotics. Zone of inhibition was measured and classified as sensitive, intermediate and resistant strains. Those organisms which were resistant to at least 3 or > 3 groups of antibiotics were considered as MDR (Magiorakos et al., 2012).

ESBL Detection: For the detection of ESBL, disk diffusion method used. Microbes were inoculated in Mueller-Hinton agar with antibiotic concentration of ceftazidime 30 microgram(µg)/ceftazidime-clavulanate 30µg/10µg and cefotaxime 30µg/cefotaxime-clavulanate 30µg/10µg and incubated for 16-20 hours in 35°C ± 2°C; ambient air. ESBL considered when a ≥ 5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate vs. the zone diameter of the agent when tested alone (CLSI., 2007).

MBL Detection: Combination disk diffusion method implemented. Microbes were inoculated onto Mueller-Hinton agar using two imipenem 10µg disks, one with 292µg EDTA placed 25mm apart. An increase in zone diameter of >4 mm around the Imipenem-EDTA disk compared to that of the Imipenem disk alone was considered positive for an MBL (Franklin et al., 2006).

MRSA Detection: Disk diffusion method used. Staphylococcus were inoculated onto Mueller-Hinton agar with cefoxitin 30µg disk and incubated for 16-18 hours in temperature 33°C-35°C. MRSA positive reported if the zone of inhibition was ≤ 21mm (CLSI., 2007).

Culture Positive Criteria:

Route of collection	Colony Count
Supra Pubic Aspirations	Urinary pathogens in any number
Urethral catheter sample	>=$50x10^3$ CFU/ml
Mid-stream sample	>10^5 CFU/ml

Conduction of the study approved from ethics and research committee of TUTH. Data's were recorded in a self-designed form and statically evaluated using Microsoft Excel 2016.

RESULTS AND DISCUSSION

Total of 155 culture positive cases, females were more affected with UTI (n=114, 73.5%) than males (n=41, 26.5%) (Figure 1). Gram-negative bacteria were the main cause of UTI (73%). Major gram-negative bacteria isolated was *E. coli* (53%) followed by *Klebsiella pneumonia* (7%), *Pseudomonas aeruginosa* (3%). Gram-positive organism comprises 27% of which major isolation was of *Enterococcus faecalis* (13%) followed by *Staphylococcus aureus* (11%.) and *Staphylococcus saprophyticus* (3%) (Figure 2).

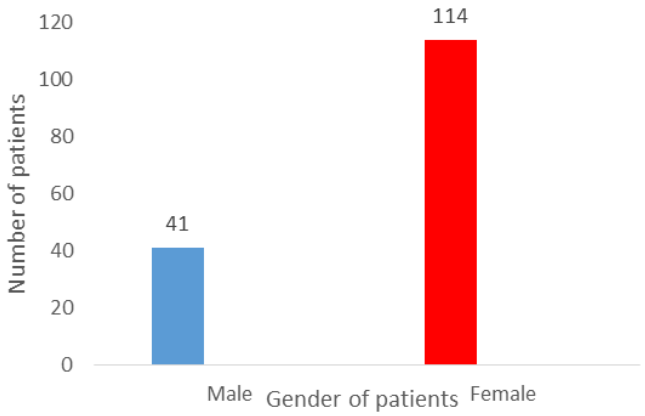

Figure 1. Gender classification of patients.

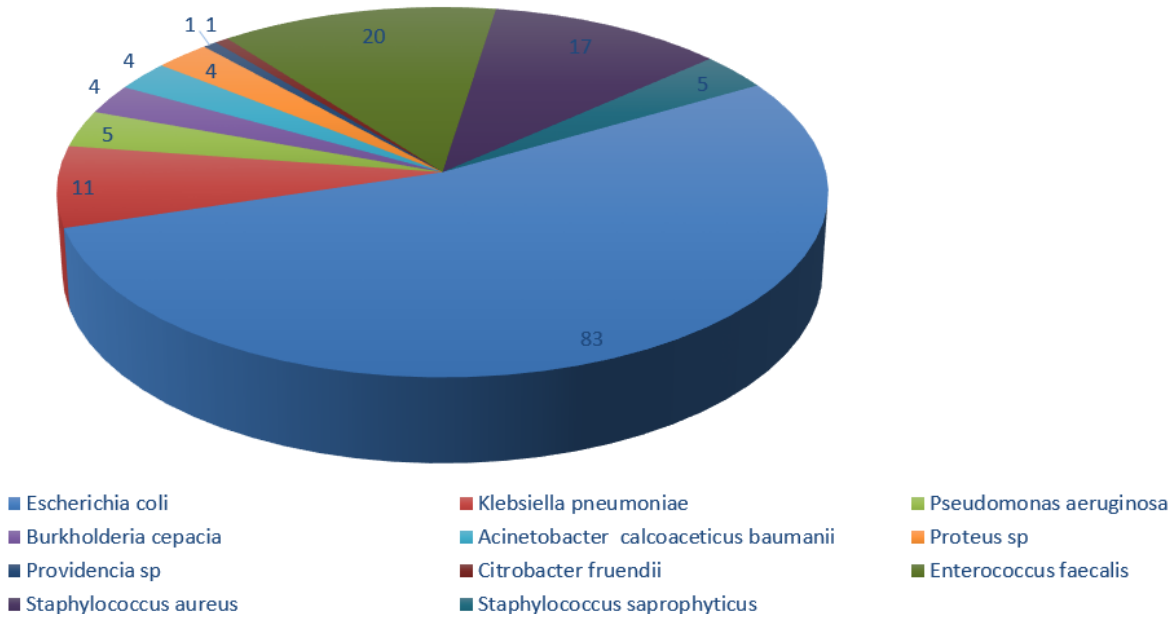

Figure 2. Pie-chart showing number and percentage of causative agents of UTI.

Majority of the micro-organisms isolated in culture were Multi-Drug Resistant (MDR) (51.94%) (Figure3). Among staphylococcus aureus, MRSA (21.41%) were isolated (Figure 4). Resistant to the major first-line antibiotics has observed. Gram-negative bacteria were found to be more sensitive to Polymyxin B, Colistin Sulphate except Burkholderia Cepacian followed by Imipenem, Amikacin, Levofloxacin, Nitrofurantoin whereas Tigecycline was found to be 100% sensitive. Gram-positive isolates were most sensitive to Vancomycin, Tigecycline, Teicoplanin followed by Gentamycin, levofloxacin, and Nitrofurantoin. All MDR bacterial isolates were 100% sensitive to Tigecycline (Table 1). The high figure of ESBL (33.92%) and MBL (3.57%) observed among Gram-negative isolates (Figure 5).

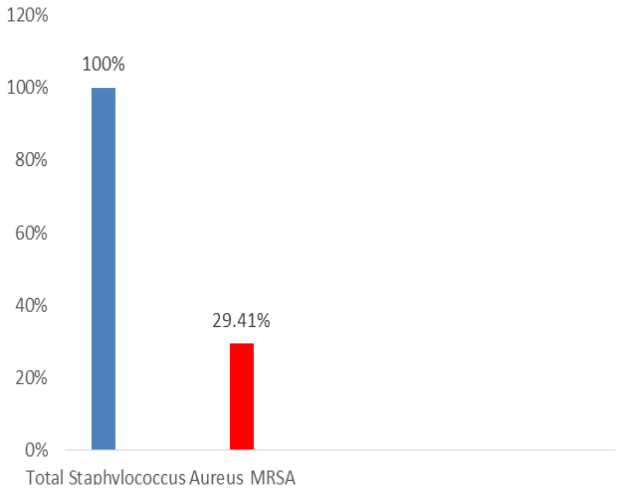

Figure 4. Percentage of Methicillin Resistant Staphylococcus Aureus (MRSA)

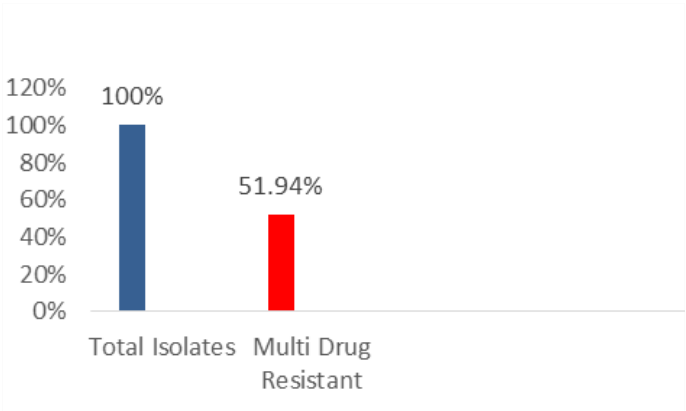

Figure 3. Percentage of multi drug resistant organism's growth on culture and sensitivity

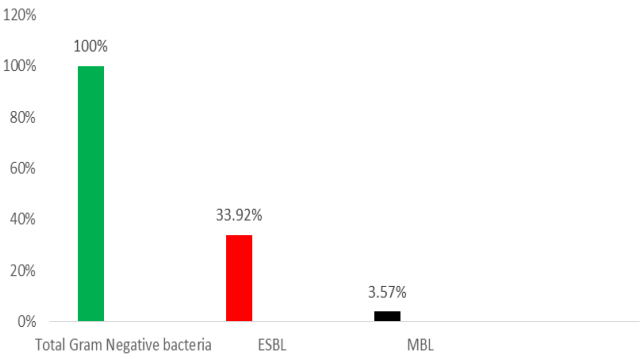

Figure 5. Percentage of Extended Spectrum β -lactamase (ESBL) and Metallo β-lactamase (MBL) among Gram negative isolates

Table 1. Sensitivity of the Micro-Organisms with first-line and second- line antibiotics

Antibiotics		Causative Micro-Organisms					
		Escherich-ia coli (n=83)	*Entero-coccus faecalis* (n=20)	*Staphylo-coccus aureus* (n=17)	*Klebsiella pneumoni-ae* (n=11)	*Pseudo-monas aeruginosa* (n=5)	*Proteus sp* (n=4)
Amoxicillin	Sensitive	3	13	2	0		0
	Resistant	80	7	15	11		4
Cefixime	Sensitive	32			6		4
	Resistant	51			5		0
Cephalexin	Sensitive	7		10	3		1
	Resistant	76		7	8		3
Ceftazidime	Sensitive	36			6	2	4
	Resistant	47			5	3	0
Ceftriaxone	Sensitive	36			6		4
	Resistant	47			5		0
Cefoperazone	Sensitive	0			0	0	
	Resistant	83			11	5	
Cefepime	Sensitive	10			2	2	
	Resistant	73			9	3	
Cefoperazone+ Sulbactam	Sensitive	25			4	3	
	Resistant	58			7	2	
Cotrimoxazole	Sensitive	38		13	6		1
	Resistant	45		4	5		3
Levofloxacin	Sensitive	48	6	16	7	3	3
	Resistant	35	14	1	4	2	1
Norfloxacin	Sensitive	37	6	12	6	2	3
	Resistant	46	14	5	5	3	1
Amikacin	Sensitive	75			10		4
	Resistant	8			1		0
Nitrofurantoin	Sensitive	75		16	3		0
	Resistant	8		1	8		4
Ampicillin+ Sulbactam	Sensitive	7			0		
	Resistant	76			11		
Piperacillin+ Tazobactam	Sensitive	27			7	3	
	Resistant	56			4	2	
Piperacillin	Sensitive	0			0	0	
	Resistant	83			11	5	
Tigecycline	Sensitive	83	20	17	11		
	Resistant	0	0	0	0		
Chloramphenicol	Sensitive	73			7		
	Resistant	10			4		
Colistin sulphate	Sensitive	81			11	5	
	Resistant	2			0	0	
Polymyxin B	Sensitive	83			11	5	4
	Resistant	0			0	0	0
Imipenem	Sensitive	75			10	4	
	Resistant	8			1	1	
Vancomycin	Sensitive		20	17			
	Resistant		0	0			
Doxycycline	Sensitive		18				
	Resistant		2				
Teicoplanin	Sensitive		20	15			
	Resistant		0	2			
Gentamycin	Sensitive			17		4	
	Resistant			0		1	
Cloxacillin	Sensitive			11			
	Resistant			6			

Antibiotics		Causative Micro-Organisms					
		Escherichia coli (n=83)	*Enterococcus faecalis* (n=20)	*Staphylococcus aureus* (n=17)	*Klebsiella pneumoniae* (n=11)	*Pseudomonas aeruginosa* (n=5)	*Proteus sp* (n=4)
Tobramycin	Sensitive			15			
	Resistant			2			
Amoxicillin+ clavulanic acid	Sensitive			2			
	Resistant			15			
Clindamycin	Sensitive			16			
	Resistant			1			

In our study, gram-negative bacteria (80.3%) were the major cause of UTI. *E. coli* isolated in (53%) followed by *Enterococcus faecalis* (13%), *Staphylococcus aureus* (11%), *Klebsiella pneumonia* (7%). A study done by (Joshi et al., 2016) found *E. coli* (66.7%) followed by *Enterococcus* (7.55%) and *Staphylococcus* (6.60%) causing UTI. This study was similar to our study by the prevalence of major uropathogens, but variation in percentage might be due to different places of studies. In another study by (Acharya et al., 2011), *E. coli* (68.77%) was a major pathogen of UTI followed by *Enterococcus* (13.92%) which was nearly equal to the percentage as found in our study *Enterococcus* (13%).

The study undertaken showed the high prevalence of MDR strains (51.94%) in UTI causing microbes. Similar results of high prevalence of MDR were seen in other studies (Baral et al., 2012; Ali et al., 2016) of 41.1% and 59% respectively. In another study by (Niranjan et al., 2014) most of the isolates in urine culture were sensitive to amikacin (82.6%), piperacillin-tazobactam (78.2%), nitrofurantoin (82.1%) and imipenem (98.9%) and sensitivity to ampicillin, cefuroxime, ceftriaxone, norfloxacin, ciprofloxacin varied between 11%-25%. The study was comparable to our study on regard to the pattern of antibiotic sensitivity. Our study also reveled most of the isolated causative microbes of UTI were sensitive to imipenem, amikacin, nitrofurantoin, piperacillin-tazobactam and developed resistant to ampicillin, ceftriaxone, and norfloxacin.

The most common cause of UTI was E. coli. All thepathogens were sensitive to tigecycline. Gram-negative bacteria were sensitive towards polymyxin-B, amikacin, imipenem, nitrofurantoin, and norfloxacin whereas gram-positive were sensitive for vancomycin, gentamycin, levofloxacin, and nitrofurantoin. A national wise review on the protocol for empirical treatment of UTI observed due to the trend of developing resistant to commonly used antibiotics.

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