Urinary tract Infection in Female Patients Visiting Tertiary Care Hospital and Determination of ESBL Producing MDR Isolates

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ABSTRACT

Objectives: The main purpose of this study was to assess the urinary tract infection (UTI) in female patients and determine the extended spectrum of beta-lactamase producers among MDR isolates.

Methods: A three-month hospital-based cross-sectional study was conducted from Marchto April 2022 at the Microbiology laboratory of Alka hospital, Lalitpur. Following the standard protocol, urine samples were taken for culture and identification. Urine samples were cultured using a semi-quantitative approach and antimicrobial susceptibility test was performed by disc diffusion method.

Results: Out of 493 female urine samples examined, significant growth was found in 106 samples. The positive isolates belonged to 5 different species. Out of them *E.coli* (77.36%) was the major pathogens isolated followed by *Klebsiella pneumoniae* (6.61%), *Klebsiella oxytoca* and *Pseudomonas aeruginosa* (1.88%) as Gram negative bacteria. And the most common Gram positive bacteria was found to be *Staphylococcus aureus* (12.26%). The most effective drug for Gram negative isolates was Amikacin (93.396%) followed by Nitrofurantoin (91.505%). The least effective drug against Gram negative bacteria was found to be Cefexime (64.16%) and Amoxicillin (58.49%). And for Gram positive isolates Ceftazidime (100%) and Amikacin (100%) were the choice of drug showing high susceptibility. The least effective drug used against Gram positive isolates tends to be Cloxacillin (15.38%). Out of 106 isolates 63 were found to be multidrug resistant among which 31 of them were ESBL producers confirmed by cephalosporin/clavulanate double disc synergy method. The urinary tract infection was most common in the age group 20-30 (20.75%) followed by age group 30-40 (16.98%).

Conclusion: UTI was least common among age groups 0-10 and 80-90 years. Bacterial isolates causing UTI had variation in the antibiotic susceptibility pattern with resistance against commonly used antibiotics which is a serious concern.

Keywords: Microbe, Urinary tract infection, urine, multidrug resistant, ESBL

INTRODUCTION

Urinary tract system consist of kidney, ureters, bladder & urethra from where UTI begins (Ahmed and Ghadeer, 2013). The colonization and proliferation of microorganisms such as bacteria and fungus within the urinary tract causes urinary tract infection. The presence of bacteria in urine is known as bacteriuria, and presence of fungi in urine is known as fungal infection. Urine passes through your urinary tract without being contaminated. Bacteria can enter the urinary tract from the outside, producing infection and irritation. The presence of bacteria in urine is known as bacteriuria (Dynamed, 1995).

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UTI is a disease caused mainly by bacteria in genitourinary tract, which spreads from the renal cortex to the kidney and urethra. UTI is most frequently caused infection which affects human and creates public health problem (Bonkat *et al*, 2017). UTIs are categorized as Upper Urinary Tract Infection: pyelonephritis, Ureterititis and Lower Urinary Tract Infection: cystitis, urethrarititis (Bailey and scotts, 2017).

Urinary tract infections are caused by both gram negative and gram positive bacteria. *Escherichia coli* is one of the most common bacteria causing Urinary Tract Infection followed by *Klebsiella* species, *Proteus mirabilis*, *Enterobacteriaceae*, *Staphylococcus aureus* and many more. These bacteria contains virulence factor which makes infection more severe by invading and multiplying in bladder (Forbes *et al*, 2002).

Antibiotics are antimicrobial substances that are effective against microorganisms. Resistance to more than three antimicrobial drugs from different structural classes is known as multidrug resistance (CDC, 2006). Multidrug resistance in bacteria is caused by the accumulation of genes, each of which codes for resistance to a specific agent, on resistance (R) plasmids or transposons, and/or the action of multidrug efflux pumps, each of which may pump out more than one drug type (Hiroshi, 2010). The commonly prescribed antibiotics for UTI are Nitrofurantoin, aminoglycosides, cephalosporin, etc.

The ESBL are enzymes which are able to inactivate variety of beta-lactam drugs, with broad spectrum penicillin's, third generation cephalosporin and monobactams which is inhibited by clavulanic acid (Rice, 1999). Time taken for detection and reporting of ESBL production by Gram negative bacteria leads to prolonged hospital stay also increases mortality morbidity and health care cost (Kollef, 2003). ESBL usually plasmid mediated beta lactamases, most commonly found in *Klebsiella pneumoniae Escherichia coli* and other Gram negative bacilli (Kaye, 2001). ESBLs are divided into various classes, each with a different evolutionary history. With approximately 150 members, the mutants of TEM and SHV-lactamases are the largest groups with CTM-X. ESBL is most common in member of *Enterobacteriaceae* family. In recent years, there has been a rise in the occurrence of ESBL (Ahmad *et al,* 2010).

MATERIALS AND METHODS

This study was conducted in the Microbiology laboratory of Alka hospital, lalitpur, from March 2022 to April 2022. During this period, a total of 493 samples were collected from female patients suspected of UTI of all age groups. During a macroscopic analysis of a urine sample, the color appearance and turbidity were examined and reported (Awasthi TR, 2016). Urine samples were cultured using a semi-quantitative approach on Blood agar (BA) and MacConkey agar (MA) plates using a standard calibrated loop. The plates were then incubated aerobically for 24 hours at 37°C. The isolates were identified using routine conventional methods based upon microscopic findings, colony morphology and biochemical properties according to standard protocol (Cheesebrough 2006). The number of bacteria colonies on the culture plates was counted. The antimicrobial susceptibility test should be perform in all positive Urine sample by using disc diffusion method. Molten sterile Muller Hinton agar was poured over base plates in sterile standard petri plates. By using sterile cotton swab lawn culture is made with the respective test organisms. Different antibiotic disc should be placed over it. The plates should be incubated in an upright position at 37°C for 24 hours. The diameter of inhibition zones are measured in mm and the results are recorded. Inhibition zones with a diameter less than 12 mm are considered as having resistance toward that antibiotic. Diameters between 12 and 16 mm are considered moderately Sensitive, Based on susceptibility pattern of isolates, bacteria showing resistance to more than 3 different classes and structures are known to be multidrug resistance (CLSI, 2011).

Criterion for Multidrug Resistance

The resistance to three or more structural classes was set as the defining condition for an isolate to be multidrug resistant in this investigation.

Screening and confirmation of ESBL producing isolates

For For screening test, Cefriaxone (30µg), Ceftazidime (30µg),cefexime (30µg), were placed onto the inoculated media and incubated for 24hours at 37º C. Isolates showing Ceftriaxone <25mm, Ceftazidime <22mm, cefexime < 15mm were suspected as possible ESBL producers, according to CLSI guideline 2016. Bacterial strains that passed the

screening test were given a phenotypic confirming test. The CLSI confirmatory test were performed using The Double Disc Synergy Test. On Muller Hinton Agar using a typical disc diffusion method. On either side of one with clavulonic acid and a disc with cephalosporin i.e cefotaxime (30 per disc) and ceftazidime (30 per disc) was applied sidewise. Depending on the species, the distance between clavulonic acid and third generation cephalosporin was altered 20cm (center to center) or 15 mm (edge to edge) (lather *et al*., 1988; Coudron in *et al*., 2003). The key like appearance in agar is thought to be confirmed indicative of ESBL production.

RESULTS

Bacterial culture

The total sample of 493 mid-stream urine were collected with proper instruction given to patient. The collected samples were runned in microbiology laboratory of Alka hospital using standard techniques. From total sample 21.5% showed significant growth and 78.50% showed no growth from given sample (Figure 1).

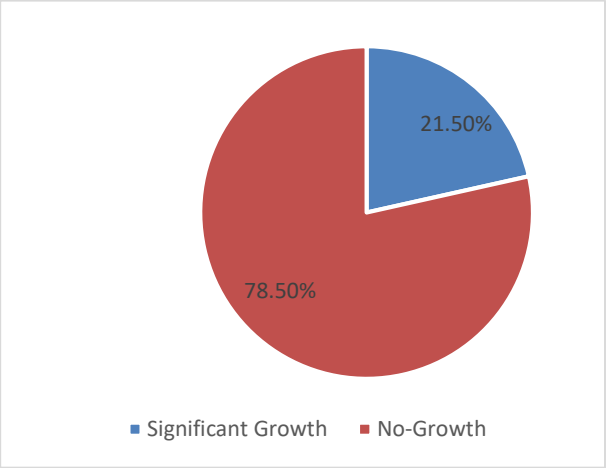

Figure 1: Pattern of culture result

Age wise distribution of significant growth

Antimicrobial From total 493 sample processed a total of 106 midstream urine samples revealed substantial bacteriuria, whereas the remaining 387 showed no growth. The age group 20-30 years had the most significant bacterial growth. Followed by age group of 30-40. The less growth was seen in age group of 0-10 and age group of 80-90, followed by age group 10-20 and age group 50-60 as shown in table 1.

Table 1: Age wise distribution of significant bacteria bacteriuria

Age group	Significant growth of bacteria	
	Number	Percentage (%)
0-10	3	2.833
10-20	5	4.716
20-30	39	36.790
30-40	23	21.698
40-50	10	9.433
50-60	9	8.490
60-70	5	4.716
70-80	9	8.490
80-90	3	2.833
Total	**106**	**100**

Growth pattern of bacterial isolates

From total of 106 isolates belonging to 5 different species were isolated from mid -stream urine sample. The most common bacteria reported were E.coli (77.36%) followed by S.aureus (12.26%), K.pneumonia (6.607%), P.aeroginosa (1.88%), and K.oxytoca (1.88%) (Table 2).

Table 2: Growth pattern of bacterial isolates

Bacteria	Total number	Percentage (%)
Escherichia coli	82	77.36
Staphylococcus aureus	13	12.26
Klebsiella pneumonia	7	6.607
Klebsiella oxytoca	2	1.88
Pseudomonas aeruginosa	2	1.88
Total	106	100

Antibiotic susceptibility pattern of Gram negative bacteria

Among the commonly used antibiotics against 93 Gram negative isolates, Nitrofurantoin was drug of choice showing susceptibility (93.60%), followed by Amakicin (92.50%), cefetriazone (59.13978%), norfloxacin (54.85%), ceftazidime (53.76%), ciprofloxacin (54.90%), cotrimoxazole (45.16%), amoxicillin (34.40%), cefexime (35.50%), nalidixic acid (27.95%) (Table 3).

Table 3: Antibiotic susceptibility of Gram negative isolates

Antibiotics used	Total no. of isolate	Susceptibility Pattern			
		Susceptible		Resistance	
		No	%	No	%
Ceftriaxone	93	55	59.14	38	40.86
Nitrofurantoin	93	87	93.60	6	6.45
Cefexime	93	33	35.50	60	64.51
Co-Trimoxazole	93	42	45.16	51	54.84
Amikacin	93	86	92.50	7	7.53
Ciprofloxacin	93	51	54.90	42	45.17
Ceftazidime	93	50	53.76	43	46.24
Amoxycillin	93	32	34.40	61	65.60
Nalidixic acid	93	26	27.95	67	72.04
Norfloxacin	93	51	54.85	42	45.16

Table 4: Antibiotic susceptibility of Gram positive isolates

Antibiotics used	Total no. of isolate	Susceptibility Pattern			
		Susceptible		Resistance	
		No	%	No	%
Ceftriaxone	13	13	100	0	0
Nitrofurantoin	13	10	76.93	3	23.07
Co-Trimoxazole	13	6	46.15	7	53.85
Amikacin	13	13	100	0	0
Ceftazidime	13	12	92.30	1	7.69
Cloxacillin	13	2	15.38	11	84.61
Norfloxacin	13	12	92.30	1	7.69
Cephalexin	13	9	69.23	4	30.77

Antibiotic susceptibility pattern of Gram positive bacteria

Among the commonly used antibiotics against 13 Gram positive isolates, Cefetriazone and Amikain was drug of choice showing susceptibility (100%), followed by Ceftazidime and norfloxacin (92.30%), Nitrofurantoin (76.93%) Cephalexin (69.23%) cotrimoxazole (46.15%), cloxacillin (15.38%) (Table 4).

Distribution of MDR gram and gram negative isolates

Multidrug drug resistance was shown by 64 isolates of urinary tract infection out of 106 isolates (60.377%). Among 93 Gram negative bacteria isoloateed 58 showed MDR which is (62.364%) in total. Higher multidrug resistance was showed by *E. coli* 51/58(87.93%) followed by *K. pneumonia* 6/7 (84.71%), *K. oxytoca* (50.0%) and *P. aeruginosa* (50.0%). And out of 13 Gram positive bacteria, *Staphylococcus* which were isolated showed 30% (4/13) MDR being resistance to more than 3 classes of antibiotics (Table 5).

Table 5: Distribution of MDR gram positive and gram negative isolates

Bacteria		Total isolates	Non-MDR	Total MDR	
				Number	(%)
Gram +ve (*S. aureus*)		13	9	4	30.76
Gram -ve	***E. coli***	82	31	51	62.19
	K. pneumoniae	7	1	6	85.71
	K. oxytoca	2	1	1	50.0
	P. aeroginosa	2	1	1	50.0

ESBL Producing organism among urine isolates

Among 98 MDR of Gram negative isolates, the screening test showed total of 59 ESBL producer. Among 59 isolates screened for ESBL 31 showed ESBL positive. ESBL producing *E. coli* were higher in number followed by *K. pnemoniae.* The confirmation was done by using cephalosporin/clavulanate combination double disc synergy method, 31/59 bacterial isolates gave positive confirmatory test for ESBL showing key like structure. ESBL

was found in only MDR isolates. Among 93 gram negative isolates only 59 showed Multi-drug resistance towards three or more than three drugs. All of the 59 isolates showed resistance to third generation cephalosporin, suspecting ESBL producing organisms. With the help of double disc synergy the positive isolated showed key like zone of inhibition. It detected 31 ESBL positive isolates (Table 6).

Table 6: Distribution of ESBL and non ESBL producer

Organism	Screening positive	ESBL positive
E. coli	51	28
K. pneumonia	6	3
K. oxytoca	1	0
P. aeruginosa	1	0

DISCUSSION

This study was carried out only in female as the incidence rate in female is higher than in male and it is one of the problematic infection in women. Females experiences one UTI case by the age 30 because of their anatomical structure. It is one of the problematic cases all around the world. The number of cases increases day by day. Following the same trend for urine culture, growth rate also was higher among female, similar results were reported by (Baral *et al,* 2012), (Ghimkre, 2013) and (Sharma, 2014).

In this study total 493 female mid-stream urine were collected from patient visiting Tertiary care hospital. Among all samples 387 (78.49%) showed no growth and 106 (21.501%) sample showed significant growth. Similar study was done by Sharma and Nerurkar (Nerurkar *et al,* 2012), (Sharma *et al,* 2013). Similar low positive results (27.3%) were observed by (Awasthi *et al*, 2013). The low positive growth rate observed in this study might be due to sample from patients under treatment or of patients with regular check-up only.

Highest growth percentage was found in age group of 20-30. This study also correlates with many other studies (Dhakal *et al,* 2002), (Dhungel, 2013), Gautam *et al,* 2012). Female anatomy, pregnancy, increased frequency of sexual intercourse, and use of diaphragm for birth control, environmental factors, hygiene practice, menstrual cycle etc may all be predisposing factors for UTI in females in the 20-30 age category (Epp *et al,* 2010), (Franz and Walter, 1999). These findings were consistent with findings of previous studies done by (Bhatt *et al*, 2012) and (Ghimire, 2013). Where significant growth was found higher in age group 21-30.

Among total 106 isolates, Gram staining revealed that 93 (87.73%) were Gram negative bacilli, conformed by biochemical tests and 13 (12.26%) were Gram positive cocci conformed by catalase and coagulase test. This result also correlates with the study done by (Maji *et al*, 2012; Rijal *et al*, 2012; Thapa *et al*, 2013). From this study and the study done by other can conclude high incidence of UTI is caused by gram negative bacteria in comparison to gram positive bacteria.

Among Gram negative isolates *E.*coli was most predominant followed by *klebsiella* species. This investigation also matched the findings of other researchers (Chakupurakal *et al*, 2010; Gautam *et al*, 2013; Thakur *et al*, 2013; Thapa *et al*, 2013). This may be due to use of P fimbriae by *E. coli* to bind uroepithelial cells in the urinary tract and colonize the bladder. Because *E.coli* bacteria come from the feces, they can easily colonize the urethra and move up the urinary tract to the bladder and kidneys. The virulent properties & Enterobacteriaceae such as adhesion, production of capsule, lipopolysaccharides Sacreted Autotransporter Toxin (SAT) & siderophores contribute them as major uropathogenes throughout the world (Jacobson *et al*, 2008; Morra *et al*, 2008). This study shows second most common pathogens were *Staphylococcus aureus* which is gram positive bacteria. Similar result was seen in the study done by (Ghimire and Rijal, 2013). *Staphylococcus aureus* reaches kidney through blood stream posing complications.

Amikacin and Nitrofurantoin were found to be the most efficient antibiotics against 86.9% of Gram negative bacteria. It was also effective against Gram positive bacteria. This conclusion was backed up by findings of other (Gautam *et al*, 2013; Rai *et al*, 2008; Thapa *et al*, 2013). Because Amikacin can dodge most of the antibiotic-inactivating enzymes that generate antibiotic resistance in bacteria, the majority of the bacteria were vulnerable to it (Lister, 2009). The least effective drug against gram negative bacteria were Nalixidic acid (69.811), Cefexime (64.159%), Amoxicillin (58.49%), Co-Trimoxazole (540717%). And for gram positive bacteria Cloxacillin was less effective drug as the

resistance percentage was (84.61%). The effective drug to treat gram positive isolates were Ceftriaxone and amikacin with 100% susceptibility. Amoxicillin and Nalixidic acid are not prescribed to treat UTI because it was least effective and this result were similarly observed by Thakur *et al,* (2013). Resistance to, many drug has been common and most complicated case nowadays. Resistance to antibiotics in uropathogens is on the rise all around the world, and it's directly related to antibiotic use and misuse (Quadeer *et al*, 2013). In this study, among total 106 isolates, MDR isolates counted for 63/106(59.43%). Out of total 93 gram negative isolate 59/93(63.44%) were found to be multidrug resistance and among 13 Gram positive bacteria Multidrug resistance was 4/13(30.76%). In this study *Escherichia coli* were found to be high among MDR isolates 51/63 (80.952%). It can be due to high use of antibiotics. Taking high dose medicines without proper instruction or prescription can also lead to MDR isolates.

The isolates resistance to cefexime, ceftazidime, ceftriazone were screened for ESBL production. In this study Out of 63 MDR, *E. coli* (51) *K. pneumoniae* (6), *K. oxytoca* (1), *P. aeroginosa* (1), they were showing resistance to cefexime, ceftriaxone, ceftazidime indicating suspension of ESBL production and were suspected for ESBL production. Out of which 31/59 showed ESBL confirmed by Clavulanic acid. In my study *klebsiella* was in second position in ESBL production though others study showed as majority of ESBL production (Dalela *et* al, 2012). This result is in harmony with the findings by Jonath, 2005; Majda et al, 2013; Shahla and Samaneh, 2010).

CONCLUSION

The incidence of UTI was found to be 21.501% in females attending Tertiary care hospital. Gram negative organisms were predominant cause of UTI. E.coli was found more than any other organism. The Gram negative isolates were more susceptible towards Amikacin and nitrofurantoin whereas cefexime and amoxicillin was least effective among drugs used.The Gram positive isolates were highly susceptible towards ceftazidime and amakicin were as cloxacillin were least effective drug showing high resistnace.MDR was observed in 59/93 bacterial isolates. Among 59 MDR isolates 31 showed ESBL positive.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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