ZIBELINE INTERNATIONAL PUBLISHING

Asian Pacific Journal of Microbiology Research (APJMR)

DOI : http://doi.org/10.26480/apjmr.01.2018.09.17

ISSN: 2329-826X (Print)
ISSN: 2329-8251 (Online)

CODEN : APJMC

PREVALENCE AND IDENTIFICATION OF UROPATHOGENS IN EASTERN NEPAL AND UNDERSTANDING THEIR ANTIBIOGRAM DUE TO MULTIDRUG RESISTANCE AND ESBL

Sanjay Mahato[1,2*], Asmita Mahato[1], Jaybendra Yadav[3]

[1]Aasra Research and Education Academy Counsel, Biratnagar 7, Nepal
[2]Department of Microbiology, Mahendra Morang Adarsha Multiple Campus, Tribhuvan University, Nepal
[3]Department of Pathology and Microbiology, Koshi Zonal Hospital, Biratnagar, Nepal
**Corresponding author E-mail: mahato.sanjay@gmail.com*

ARTICLE DETAILS

Article History:

Received 26 June 2018
Accepted 2 July 2018
Available online 1 August 2018

ABSTRACT

This study was basically aimed at identifying the diversity of uropathogens; their drug response like multi-drug resistance (MDR) or extended spectrum β-lactamase (ESBL); and the evaluation of empirical treatment. Between January 2016 and December 2016, urine samples from patients of Koshi Zonal Hospital, Biratnagar, were collected in this cross-sectional descriptive study. Of 3666 urine samples, 414 (11.3%) samples were found to be positive with significant bacteriuria. 70.53% of prevalence was seen in female and 29.47% was in the male. Most common bacteria were *E. coli* (67.87%) followed by *Klebsiella* (14.01%) and *Pseudomonas* (13.77%) respectively. Of the total 281 *E. coli* positive isolates, 111 (39.5%) were found to be MDR while 64 (22.8%) were identified as ESBLs. Out of total 57 *Pseudomonas*, 63.16% were MDR and 5.25% were ESBLs. Of 58 *Klebsiella* samples, 56.9% were accounted as MDR and 6.9% as ESBLs. Isolates were mostly susceptible to amikacin, nitrofurantoin, and levofloxacin, but, were highly resistant to ampicillin, cefexime, and cefotaxime. Multiple antibiotic resistance (MAR) indices of bacteria indicated an alarming scenario of drug resistance. The increasing resistance to several classes of drugs and the extended spectrum is increasingly making empirical treatment ineffective ultimately leading to a high cost of treatment.

KEYWORDS

Multidrug resistance, ESBLs, Multiple antibiotic resistance indices, uropathogens.

1. INTRODUCTION

Both gram negative and gram-positive bacteria is associated with urinary tract infections (UTIs). *E. coli* is the most common causative agent of UTIs. Other organisms reported include members of the family *Enterobacteriaceae* (i.e., *Klebsiella, Proteus, Citrobacter and Enterobacter* spp.), *Enterococcus* species, *Pseudomonas* species, streptococci, staphylococci, and *Candida albicans* [1]. Multidrug resistance among such organisms is increasing and such resistance considerably limits patient treatment options [2-4]. β-lactam antibiotics are currently the main antibacterial agents for the treatment of serious infections due to *Enterobacteriaceae.* However, their random and increasing use has led to the emergence of extended spectrum β-lactamase (ESBL)-producing strains [5]. These enzymes hydrolyze all penicillins, cephalosporins and oxyimino β-lactams, but are inactive against carbapenems and cephamycins [6]. β-lactamases are classified into four classes (A, B, C, and D) based on their amino acid sequence and inactivate β-lactam antibiotics [7]. In order to overcome the β-lactamase-mediated drug resistance, β-lactam antibiotics are combined with β-lactamase inhibitors (BLI). Clavulanic acid, sulbactam, tazobactam, and avibactam are four clinically-used BLIs [8].

Gram negative bacteria like *E. coli*, *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* are among major causes of infection. The gastrointestinal tract serves as a reservoir of *Klebsiella* and is often the latent source of infections [9,10]. The selection of most appropriate antimicrobial therapy is complicated because of the great ability of *P. aeruginosa* to develop or acquire resistance to multiple classes of antimicrobials [11]. *P. aeruginosa* is integrally resistant to antibiotics largely due to multiple chromosomally encoded multidrug efflux systems as well as low membrane permeability [12]. The class A extended-spectrum β-lactamases (ESBLs) confers resistance to expanded-spectrum cephalosporins and are inhibited in vitro by clavulanic acid and tazobactam [13]. Resistance to broad-spectrum cephalosporins in *Acinetobacter baumannii* mostly results from overexpression of the natural AmpC-type enzyme or from the acquisition of ESBLs [14].

Most infections in UTIs are uncomplicated. Patients having functional, metabolic or structural abnormalities show infections of complicated nature. The etiology of complicated UTI is more diverse and often polymicrobial in nature. Specific host factors, such as the extremes of age, pregnancy, catheterization, diabetes, or spinal cord injuries, can impact on etiology [15].

As most UTIs are treated empirically, the selection of antimicrobial agents should be based on the most likely pathogen and its expected resistance pattern in that geographic area [16, 17]. That's why the periodic monitoring of causative agents of UTI and their resistance pattern in a given locality or region are essential [16]. Suitable empirical treatment of urinary tract infections (UTIs) is vital for successful treatment and prevention of complications. Patients with UTIs receiving inappropriate empirical therapy are associated with longer treatments, the frequency of hospital visits or lengths of hospital stay as well as high treatment costs.

2. MATERIALS AND METHODS

2.1 Study Area, Specimen Size, and Type

Clean catch urine samples were collected from both inpatients and outpatients of the Koshi Zonal Hospital, Biratnagar, Morang. This sample collection site was chosen because it not only covers the urban population of the city but also the distant rural population. Diversified population visits the hospital for the reason that the hospital is government owned and the treatment cost is lower. The duration of the study was one year (January 2016 to December 2016). The study was carried out in the Microbiology Laboratory of the Koshi Zonal Hospital, Morang, Nepal and AASRA Research and Education Academic Counsel, Biratnagar, Nepal. A total of 3666 clinical samples of urine were processed during the study period. Repeated sample of the same patient was not included in the study. The informed consent was taken from the patients.

2.2 Sample Collection

To avoid contamination, each patient/ individual was instructed how to collect a "clean-catch" mid-stream urine specimen by laboratory personnel. About 10 to 20 ml urine specimen was collected in a 20 mL sterile screw-capped and wide mouthed universal container. The container containing specimen was appropriately labeled with unique sample number, date, and time of collection. After collection, it was forwarded to the Microbiology laboratory of Koshi Zonal Hospital for culture and drug susceptibility testing. The specimen was analyzed within 2 hours after collection.

2.3 Sample Processing/ Culture

The samples were processed according to a previously described methodology. Only patients that presented with clinical symptoms of UTI and positive urine culture ($\geq 10^5$ CFU/mL) were studied. Significant bacteriuria was defined as colony count $\geq 10^5$ CFU/mL. Each urine sample was aseptically inoculated (in triplicate) into MacConkey agar plates (Himedia, Mumbai, India), 5% sheep blood agar (Himedia), mannitol salt agar plates (Himedia), and cetrimide agar plates (Himedia) on arrival at the laboratory. The plates were incubated aerobically at 37^0C for 18-24 hr. The colonial characteristics of bacterial isolates on the selective media plates were observed and were aseptically sub-cultured onto freshly prepared culture media plates.

2.4 Identification of Organisms

The resulting cultures/isolates were subjected to microscopical examination like Gram staining and capsule staining and appropriate biochemical tests for proper identification. Gram positive isolates were further identified by oxidase, catalase, coagulase, and optochin sensitivity tests while for the identification of Gram negative isolates different biochemical tests like oxidase, catalase, motility, indole and H_2S production, MR-VP, citrate utilization, urea hydrolysis, triple sugar iron utilization were done. The identity of bacteria was established based based on their cultural and biochemical characteristics as described in the book of Cheesbrough [18]. The identified bacterial isolates were maintained in nutrient agar slants, incubated at 37^0C for 24 hr, and subcultured periodically.

2.5 Antibiotic Susceptibility Study

Antibiotic susceptibility testing of the bacterial isolates was performed by disc diffusion method (Kirby-Bauer method) on Muller-Hinton agar (Himedia, Mumbai, India) and interpreted according to the Clinical Laboratory Standard Institute (CLSI) guidelines [19]. A homogenous suspension of 0.5 MacFarland standard of a pure colony was prepared in 5 mL of sterile normal saline (0.85% NaCl). Using a sterile swab, the bacterial suspension was evenly distributed over the entire surface of Mueller-Hinton Agar (MHA) plates. For antimicrobial testing of streptococci, 5% defibrinated sterile sheep blood was aseptically mixed to molten Mueller-Hinton Agar before plating. The antibiotic disc (Himedia, India) containing the following antibiotics was used: Amikacin (AK, 30 µg), Ampicillin (AMP, 10 µg), Cefexime (CFM, 5 µg), Cefotaxime (CTX, 30 µg), Cotrimoxazole (COT, 25 µg), Nitrofurantoin (NIT, 300 µg), Ofloxacin (OF, 5 µg), Levofloxacin (LE, 5 µg). Once the discs were applied onto MHA plates, the plates were incubated at 37^0C for 24 hr. Zone of inhibition was measured and interpreted using the standard chart and the organisms were reported as susceptible, intermediate, or resistant accordingly [19].

2.6 The criterion for Multidrug Resistance

All those isolates which demonstrated the resistance to at least one agent in three or more classes of the drug were defined as multidrug resistant (MDR) [19-21].

2.7 ESBL detection

Phenotypic – the ESBL detection was done as per the recommendation of the CLSI confirmatory procedure, by using cefotaxime (30 µg) discs alone and in combination with clavulanic acid discs. Isolates showing a zone of inhibition <27 mm to cefotaxime were considered as possible ESBL producers. Further, the ESBL producers were confirmed only if there was an increase in zone diameter of ≥5 mm in presence of cefotaxime plus clavulanic acid from cefotaxime alone [20, 22].

2.8 Multiple antibiotic resistance (MAR) index

MAR index is a number of antibiotics to which test isolate displayed resistance divided by the total number of antibiotics to which the test organism has been evaluated for sensitivity. So MAR index for each isolate was calculated as per the recommendation of Krumperman [23].

2.9 Data Analysis

The data were statistically analyzed using Statistical Package for Social Sciences (SPSS v21) software package. Data frequencies and cross tabulations were used to summarize descriptive statistics. Tables were used for data presentation. Odd ratio and adjusted odd ratio were employed in the analysis. Both bivariate and multiple logistic regressions were employed to assess the association between outcome and explanatory variables. P values <0.05 were considered statistically significant.

3. RESULTS

Of 3666 urine samples, 414 samples (11.3%) were found to be positive with significant bacteriuria. No growth was seen in 87.4% of the samples while 1.3% showed insignificant growth. Interestingly mixed growth was seen only as insignificant growth in some cases. Among 3666 individuals, 2232 (60.9%) were female and 1434 (39.1%) were male (Table 1). Mean age of the participants was 20.77 ± 0.30 years. Female participants had a mean age of 23.11 ± 0.359 (CI 22.41, 23.81) while male participant showed a mean of 17.13 ± 0.511 (CI 16.13, 18.13). Out of 414 positive samples, 70.53% of the incidence was in female (292 in number) and 29.47% was in male (122 in number). Mean age of positive patients was 23.41 ± 0.956 (CI 21.53, 25.29) years where infected female showed a mean age of 25.96 ± 1.128 (CI 23.73, 28.18) and the infected male had a mean age of 17.23 ± 1.689 (CI 13.88, 20.57).

Table 1: Odds Ratio for Prevalence of bacterial UTI.

Urine Culture	Female	Male	Total	Odd Ratio (CI)	p Value
Positive	292	122	414	1.632 (1.306-2.039)	0.001
Insignificant	35	13	48	1.836 (0.968-3.483)	0.063
No Growth	1905	1299	3204	-	-

The reference category is: No Growth
Male is redundant.

Among total participants, nearly three-fourth (74.8%) were below 30 years of age where less than 10 years of age group had the highest participants (34.6%) followed by 10-19 years of age (21.4%) and, then 20-29 years of age (18.8%). The rest of the age group were followed by a decreasing trend of the participant with the increase in age.

Of all the positive samples, 97.3% were found to be gram negative isolates. *E. coli* (67.87%) was the most prevalent isolated organism in UTI followed by *Klebsiella* (14.01%), *Pseudomonas* (13.77%) and *Enterococcus* (1.93%). The majority of infections were caused by *E. coli* (67.87%), *Klebsiella* (14.01%) viz. *Klebsiella* spp. (7.49%) and *K. pneumoniae (6.52%), Pseudomonas* (13.77%) namely *P. aeruginosa* (12.08%) and *Pseudomonas* spp. (1.69%), and *Enterococcus* (1.93%) namely *Enterococcus faecalis* (1.69%) and *Enterococcus* spp. (0.24%) (Table 2). Rest of the causative gram-negative bacteria were <1% each namely *Proteus* spp. (0.97%), *Citrobacter* spp. (0.48%), *Acinetobacter* spp. (0.24%). Such a low occurrence was also seen with gram positive bacteria like *Enterococcus* (1.93%), *Staphylococcus aureus* (0.48%) and *Streptococcus* spp. (0.24%).

Table 2: Incidence of bacterial isolates from urine samples and their multidrug resistant profile

Bacterial isolates	Number of Isolates	Population		Ocurrence (%)	Multidrug resistance Isolates number (%)	ESBL Number (%)
		Male	Female			
Escherichia coli	281	78	203	67.87	111 (39.5)	64 (22.8)
P. aeruginosa	50	16	34	12.08	34 (68)	3 (6)
Pseudomonas spp.	7	2	5	1.69	2 (28.6)	-
Klebsiella spp.	31	9	22	7.49	16 (51.6)	3 (9.7)
Klebsiella pneumoniae	27	10	17	6.52	17 (63)	1 (3.7)
Enterococcus faecalis	7	2	5	1.69	5 (71.4)	-
Enterococcus spp.	1	1	0	0.24	1 (100)	-
Proteus spp.	4	1	3	0.97	4 (100)	-
Staphylococcus aureus	2	0	2	0.48	2 (100)	-
Citrobacter spp.	2	2	0	0.48	1 (50)	-
Acinetobacter spp.	1	0	1	0.24	0	-
Streptococcus spp.	1	1	0	0.24	0	
Total	**414**	**122**	**292**	**100%**	**193 (46.6%)**	**71 (17.2%)**

Taking all the major isolates, it was established that gender was not associated with types of microbes (χ^2=1.066, df = 3, p=0.785). On the contrary, gender and infection were significantly associated (χ^2=21.837, df = 2, p<0.001). While investigating, it was found that gender was neither associated to MDR (χ^2=0.169, df = 1, p=0.681) nor ESBLs (χ^2=0.095, df = 1, p=0.776).

Out of 281 *E. coli* positive isolates, 39.5% was found to be multidrug resistant (MDR) while 22.8% was identified as ESBLs. In the case of 50 *Pseudomonas aeruginosa* isolates, 68% was MDR and 6% was ESBLs. *Pseudomonas* spp. (7 in number) whose species couldn't be identified showed only 2 isolates as MDR and none as ESBL. Hence on adding all the *P. aeruginosa* and *Pseudomonas* spp., total MDR and ESBLs were 63.16% and 5.25% respectively. *Klebsiella* as a genus accounted 56.9% MDR and 6.9% ESBLs out of 58 positive isolates (31 *Klebsiella* spp. and *K. pneumoniae* 27 in number). Out of 7 *Enterococcus faecalis,* 5 were identified as MDR. Even 1 *Enterococcus* spp. whose species wasn't established was found to be MDR. All *Proteus* spp. (4 in number) and *Staphylococcus aureus* (2 in number) were MDR. Out of 2 *Citrobacter* spp., one was MDR. More than two-third of ESBL *E. coli* (68.75%) were found up to 29 years of age. Highest ESBL *E. coli* (28.13%) was found in the age group of 20-29. 21.9% and 18.75% ESBL *E. coli* were found in <10 and 10-19 years of age group respectively (Table 3). Since *Pseudomonas* and *Klebsiella* showed very low ESBLs, the prevalence in age group couldn't be established.

Table 3: Distribution of ESBL isolates with respect to age and sex

Age	*Escherichia coli*			*Pseudomonas* spp. + *P. aeruginosa*			*Klebsiella* spp. + *K. pneumoniae*		
	Total	Female	Male	Total	Female	Male	Total	Female	Male
<10	14	5	9	1	-	1	2	2	-
10-20	12	9	3	-	-	-	1	-	1
20-30	18	15	3	1	1	-	1	1	-
30-40	6	5	1	-	-	-	-	-	-
40-50	2	1	1	1	1	-	-	-	-
50-60	4	4	-	-	-	-	-	-	-
60-70	3	1	2	-	-	-	-	-	-
70-80	4	3	1	-	-	-	-	-	-
80-90	1	1	-	-	-	-	-	-	-
90-100	-	-	-	-	-	-	-	-	-

Although age was significantly associated with infection (χ^2=25.249, df = 12, p=0.014), no relationship could be established between age and type of uropathogen isolated (χ^2=7.151, df = 6, p=0.307). Similarly, non-significant relationship of age was observed not only with ESBLs (χ^2=3.374, df = 3, p=0.337), but also with MDR (χ^2=8.947, df = 8, p=0.347). Out of 414 positive samples, even more than two-third of the incidence was seen in female (70.53%) and quite lower than one-third was in male (29.47%). The highest incidence rate was found to be 28.02% in age group of lesser than 10 years followed by the age group of 20-29 years and 10-19 years with 22.71% and 20.53% respectively (Table 4). From 30 years of age onwards, the infection rate was decreasing chronologically with the age group. Till 29 years of age, the share of infection was found to be 71.26%. An interesting observation was seen between incidence rate of age (till 29 years) and gender. In age group lesser than 10 years, the rate of infection in female (51.72%) was slightly higher than male (48.28%). While gender-wise infection share (female 62.35% and male 37.65%) of age group 10-19 years roughly in agreement with the overall gender-wise incidence rate, the infection rate in 20-29 years was surprisingly higher in female (91.49%) than male (8.51%). Though lower than 20-29 years, 30-39 years age group also showed much higher infection in female (82.5%) to male (17.5%).

Table 4: Age and Sex-wise distribution of isolates

Age	Total number of isolates	Incidence Rate (%)	Female (%)	Male (%)
<10	116	28.02	60 (51.72)	56 (48.28)
10-20	85	20.53	53 (62.35)	32 (37.65)
20-30	94	22.71	86 (91.49)	8 (8.51)
30-40	40	9.66	33 (82.5)	7 (17.5)
40-50	26	6.28	20 (76.92)	6 (23.08)
50-60	23	5.56	18 (78.26)	5 (21.74)
60-70	13	3.14	8 (61.54)	5 (38.46)
70-80	12	2.90	9 (75)	3 (25)
80-90	3	0.72	3 (100)	0 (0)
90-100	2	0.48	2 (100)	0 (0)
Total	**414**	**100%**	**292**	**122**

An interesting observation was noted down with the age group of 20-30 years where *E. coli* in female was found to be 90.77% (Table 5). The occurrence of *Pseudomonas* and *Klebsiella* in this age group also followed the similar pattern in the female population with 92.86% and 92.31% respectively. In contrast to age group 20-29, age group less than 10 years showed a higher occurrence of *Pseudomonas* and *Klebsiella* in male population i.e. 75% and 57.14% respectively and slightly falling behind to female (55%) in the case of *E. coli*. No sample tested positive with *Pseudomonas* and *Klebsiella* above 69 years of age group. On the contrary, *E. coli* was distinctively figured with 4.27% in 70-79 years age group and even showed its presence above 79 years age group.

Table 5: Distribution of major types of uropathogens with respect to age and sex

Age	*Escherichia coli*			*Pseudomonas* spp. + *P. aeruginosa*			*Klebsiella* spp. + *K. pneumoniae*		
	Total	**Female**	**Male**	**Total**	**Female**	**Male**	**Total**	**Female**	**Male**
<10	80	44	36	20	5	15	21	9	12
10-20	55	35	20	13	8	5	12	8	4
20-30	65	59	6	14	13	1	13	12	1
30-40	22	19	3	9	6	3	7	6	1
40-50	17	14	3	5	3	2	2	2	-
50-60	17	14	3	2	2	-	2	1	1
60-70	9	5	4	2	1	1	1	1	-
70-80	12	9	3	-	-	-	-	-	-
80-90	2	2	-	-	-	-	-	-	-
90-100	2	2	-	-	-	-	-	-	-

The antimicrobial susceptibilities of *E. coli, K. pneumoniae, Klebsiella* spp., *Pseudomonas aeruginosa, Pseudomonas* spp., *Enterococcus faecalis, Enterococcus* spp., and those of *Staphylococcus aureus, Citrobacter* spp., *Acinetobacter* spp., *Streptococcus* spp. are shown in Table 6.

Of all gram-negative bacteria (n=403) namely *E. coli, Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp., *Citrobacter* spp. and *Acinetobacter* spp., 97.5% were most susceptible to amikacin, 86.8% to nitrofurantoin and 79.1% to levofloxacin. Isolates were highly resistant to drugs like ampicillin (91.3%), cefexime (76.2%) and cefotaxime (80.9%). While 52.4% were cotrimoxazole resistant, 57.8% were ofloxacin sensitive. Susceptibility pattern shown by *E. coli, K. pneumoniae, Klebsiella* spp., *Pseudomonas* spp., *P. aeruginosa, Proteus* spp., *Citrobacter* spp. and *Acinetobacter* spp. to amikacin, nitrofurantoin and levofloxacin were in accordance with the overall results of our study. All these isolate types were highly resistant to ampicillin and cefexime. Only found *Acinetobacter* spp. was sensitive to cefexime. While *E. coli* and *Proteus* spp. were highly resistant to cefotaxime, all *Citrobacter* spp. and *Acinetobacter* spp. were susceptible. *Klebsiella and Pseudomonas* were evenly resistant to cefotaxime. *E. coli*, *Klebsiella, Pseudomonas, Proteus* spp., *Citrobacter* spp. were marginally sensitive to ofloxacin. Only found *Acinetobacter* was sensitive to ofloxacin. Cotrimoxazole was marginally resistant to *E. coli*, *Klebsiella*, *Pseudomonas,* and *Citrobacter. Proteus* spp. and *Acinetobacter* showed resistance to cotrimoxazole.

Table 6: Antimicrobial susceptibility pattern of isolated organisms from urine samples

Organism	Type of antibiotic	Susceptibility level		
		Susceptible	**Intermediate**	**Resistant**
E. coli	Amikacin	274 (97.5%)	-	7 (2.5%)
	Ampicillin	25 (8.9%)	-	256 (91.1%)
	Cefexime	64 (22.8%)	4 (1.4%)	213 (75.8%)
	Cefotaxime	12 (4.3%)	5 (1.8%)	264 (94%)
	Cotrimoxazole	129 (45.9%)	14 (5%)	138 (49.1%)
	Nitrofurantoin	241 (85.8%)	1 (0.4%)	39 (13.9%)
	Ofloxacin	168 (59.8%)	-	113 (40.2%)
	Levofloxacin	212 (75.4%)	4 (1.4%)	65 (23.1%)
P. aeruginosa	Amikacin	48 (96%)	-	2 (4%)
	Ampicillin	2 (4%)	-	48 (96%)
	Cefexime	5 (10%)	-	45 (90%)
	Cefotaxime	23 (46%)	-	27 (54%)
	Cotrimoxazole	15 (30%)	-	35 (70%)
	Nitrofurantoin	43 (86%)	-	7 (14%)
	Ofloxacin	25 (50%)	-	25 (50%)
	Levofloxacin	45 (90%)	-	5 (10%)
Pseudomonas spp.	Amikacin	7 (100%)	-	-
	Ampicillin	-	-	7 (100%)
	Cefexime	2 (28.6%)	-	5 (71.4%)
	Cefotaxime	3 (42.8%)	-	4 (57.1%)
	Cotrimoxazole	6 (85.7%)	-	1 (14.2%)
	Nitrofurantoin	6 (85.7%)	-	1 (14.2%)
	Ofloxacin	7 (100%)	-	-

	Levofloxacin	7 (100%)	-	-
Klebsiella spp.	Amikacin	30 (96.8%)	-	1 (3.2%)
	Ampicillin	5 (16.1%)	-	26 (83.9%)
	Cefexime	11 (35.5%)	-	20 (64.5%)
	Cefotaxime	17 (54.8%)	-	14 (45.2%)
	Cotrimoxazole	17 (54.8%)	-	14 (45.2%)
	Nitrofurantoin	29 (93.6%)	-	2 (6.4%)
	Ofloxacin	17 (54.8%)	-	14 (45.2%)
	Levofloxacin	28 (90.3%)	-	3 (9.7%)
K. pneumoniae	Amikacin	27 (100%)	-	-
	Ampicillin	3 (11.1%)	-	24 (88.9%)
	Cefexime	9 (33.3%)	-	18 (66.7%)
	Cefotaxime	13 (48.1%)	-	14 (51.9%)
	Cotrimoxazole	10 (37.0%)	-	17 (63%)
	Nitrofurantoin	24 (88.9%)	-	3 (11.1%)
	Ofloxacin	12 (44.4%)	-	15 (55.6%)
	Levofloxacin	22 (81.5%)	-	5 (18.5%)
Proteus	Amikacin	4 (100%)	-	-
	Ampicillin	-	-	4 (100%)
	Cefexime	-	-	4 (100%)
	Cefotaxime	1 (25%)	-	3 (75%)
	Cotrimoxazole	-	-	4 (100%)
	Nitrofurantoin	4 (100%)	-	-
	Ofloxacin	2 (50%)	-	2 (50%)
	Levofloxacin	3 (75%)	-	1 (25%)
Citrobacteria	Amikacin	2 (100%)	-	-
	Ampicillin	-	-	2 (100%)
	Cefexime	-	-	2 (100%)
	Cefotaxime	2 (100%)	-	-
	Cotrimoxazole	1 (50%)	-	1 (50%)
	Nitrofurantoin	2 (100%)	-	-
	Ofloxacin	1 (50%)	-	1 (50%)
	Levofloxacin	1 (50%)	-	1 (50%)
Acinetobacter	Amikacin	1 (100%)	-	-
	Ampicillin	-	-	1 (100%)
	Cefexime	1 (100%)	-	-
	Cefotaxime	1 (100%)	-	-
	Cotrimoxazole	-	-	1 (100%)
	Nitrofurantoin	1 (100%)	-	-
	Ofloxacin	1 (100%)	-	-
	Levofloxacin	1 (100%)	-	-
Total Gram negative isolates	**Amikacin**	**393 (97.5%)**	**-**	**10 (2.5%)**
	Ampicillin	**35 (8.7%)**	**-**	**368 (91.3%)**
	Cefexime	**92 (22.8%)**	**4 (1%)**	**307 (76.2%)**
	Cefotaxime	**72 (17.9%)**	**5 (1.2%)**	**326 (80.9%)**
	Cotrimoxazole	**178 (44.2%)**	**14 (3.5%)**	**211 (52.4%)**
	Nitrofurantoin	**350 (86.9%)**	**1 (0.2%)**	**52 (12.9%)**
	Ofloxacin	**233 (57.8%)**	**-**	**170 (42.2%)**
	Levofloxacin	**319 (79.2%)**	**4 (1%)**	**80 (19.8%)**
E. facealis	Amikacin	6 (85.7%)	-	1 (14.3%)
	Ampicillin	-	-	7 (100%)
	Cefexime	-	-	7 (100%)
	Cefotaxime	5 (71.4%)	-	2 (28.6%)
	Cotrimoxazole	3 (42.9%)	-	4 (57.1%)
	Nitrofurantoin	6 (85.7%)	-	1 (14.3%)
	Ofloxacin	3 (42.9%)	-	4 (57.1%)
	Levofloxacin	5 (71.4%)	-	2 (28.6%)
Enterococcuus spp.	Amikacin	1 (100%)	-	-
	Ampicillin	-	-	1 (100%)
	Cefexime	1 (100%)	-	-
	Cefotaxime	1 (100%)	-	-
	Cotrimoxazole	1 (100%)	-	-
	Nitrofurantoin	-	-	1 (100%)
	Ofloxacin	-	-	1 (100%)
	Levofloxacin	1 (100%)	-	-
S. aureus	Amikacin	2 (100%)	-	-
	Ampicillin	-	-	2 (100%)

	Cefexime	-	-	2 (100%)
	Cefotaxime	-	-	2 (100%)
	Cotrimoxazole	-	-	2 (100%)
	Nitrofurantoin	2 (100%)	-	-
	Ofloxacin	-	-	2 (100%)
	Levofloxacin	2 (100%)	-	-
Streptococcus spp.	Amikacin	1 (100%)	-	-
	Ampicillin	-	-	1 (100%)
	Cefexime	1 (100%)	-	-
	Cefotaxime	1 (100%)	-	-
	Cotrimoxazole	-	-	1 (100%)
	Nitrofurantoin	1 (100%)	-	-
	Ofloxacin	1 (100%)	-	-
	Levofloxacin	1 (100%)	-	-
Gram Positive isolates	**Amikacin**	**10 (90.9%)**	**-**	**1 (9.1%)**
	Ampicillin	**-**	**-**	**11 (100%)**
	Cefexime	**2 (18.2%)**	**-**	**9 (81.8%)**
	Cefotaxime	**7 (63.6%)**	**-**	**4 (36.4%)**
	Cotrimoxazole	**4 (36.4%)**	**-**	**7 (63.6%)**
	Nitrofurantoin	**9 (81.8%)**	**-**	**2 (18.2%)**
	Ofloxacin	**4 (36.4%)**	**-**	**7 (63.6%)**
	Levofloxacin	**9 (81.8%)**	**-**	**2 (18.2%)**

Of all gram-positive bacteria (n=11) namely *Enterococcus* spp., *Staphylococcus aureus* and *Streptococcus,* 90.9% were most susceptible to amikacin, 81.8% to nitrofurantoin and levofloxacin and 63.6% to cefotaxime. Isolates were highly resistant to drugs like ampicillin (100%), cefexime (81.8%), cotrimoxazole (63.6%) and ofloxacin (63.6%). A majority of *Enterococcus* was sensitive to amikacin, cefotaxime, nitrofurantoin, and levofloxacin, while resistant to ofloxacin, cotrimoxazole, and cefexime. All of the *Enterococcus* were resistant to ampicillin. All the strains of *Staphylococcus aureus* and *Streptococcus* were resistant to ampicillin and cotrimoxazole while sensitive to amikacin, nitrofurantoin, and levofloxacin. While all *S. aureus* was resistant to cefexime, cefotaxime, and ofloxacin, *Streptococcus* was sensitive to these drugs.

All the ESBLs isolated in our study which was gram negative showed 100% resistance to ampicillin, cefexime, and cefotaxime (Table 7). While *E. coli* was highly resistant to cotrimoxazole (89.1%), ofloxacin (87.5%) and levofloxacin (81.3%), *Klebsiella* and *Pseudomonas* were 100% resistant to cotrimoxazole, ofloxacin, and levofloxacin. Eventually, ESBLs were highly sensitive to amikacin and nitrofurantoin.

Table 7: Antimicrobial susceptibility pattern of ESBL isolates from urine samples

Antibiotics	**ESBL**					
	***E. coli* (n=64)**		***Klebsiella spp. + K. pneumoniae* (n=4)**		***Pseudomonas aeruginosa* (n=3)**	
	Sensitive (%)	**Resistant (%)**	**Sensitive (%)**	**Resistant (%)**	**Sensitive (%)**	**Resistant (%)**
Amikacin	62 (96.9)	2 (3.1)	4 (100)	0	2 (66.7)	1 (33.3)
Ampicillin	0	64 (100)	0	4 (100)	0	3 (100)
Cefexime	0	64 (100)	0	4 (100)	0	3 (100)
Cefotaxime	0	64 (100)	0	4 (100)	0	3 (100)
Cotrimoxazole	7 (10.9)	57 (89.1)	0	4 (100)	0	3 (100)
Nitrofurantoin	53 (82.8)	11 (17.2)	3 (75)	1 (25)	3 (100)	0
Ofloxacin	8 (12.5)	56 (87.5)	0	4 (100)	0	3 (100)
Levofloxacin	12 (18.7)	52 (81.3)	0	4 (100)	0	3 (100)

Multiple antibiotic resistance (MAR) indices of bacteria revealed that only 6.4% (n=18) of *E. coli,* 3.5% (n=2) of *Pseudomans,* 12.1% (n=7) of *Klebsiella* were susceptible to all the eight tested drugs with MARI of zero while none were resistant to all the drug (Table 8). Of all 281 *E. coli*, 16 (5.7%) were resistant to 1 drug (MARI = 0.125), 43 (15.3%) were resistant to 2 drugs (MARI = 0.25), 57 (20.3%) were resistant to 3 drugs (MARI = 0.375), 53 (18.9%) were resistant to 4 drugs (MARI = 0.5), 38 (13.5%) were to 5 drugs (MARI = 0.625), 45 (16.0%) were to 6 drugs (MARI = 0.75) and 11 (3.9%) were resistant to 7 drugs (MARI = 0.875). 29.8% of *Pseudomonas* were resistant to 4 drugs (MARI = 0.5), 22.8% to 5 drugs (MARI = 0.625) and 19.3% to 3 drugs (MARI = 0.375). Though not high 7.0% of *Pseudomonas* showed resistant to 6 drugs (MARI = 0.65) and 1.8% to 7 drugs (MARI = 0.75). 31.0% of *Klebsiella* were resistant to 4 drugs (MARI = 0.5), 10.3% to 5 drugs (MARI = 0.625), 12.1% to 6 drugs (MARI = 0.75), 13.8% to 2 drugs (MARI = 0.25), 10.3% to 3 drugs (MARI = 0.375) and 8.6% to 1 drug (MARI = 0.125). Eventually 1 strain (1.7%) was found to be resistant to 7 (MARI = 0.875) drugs. *Enterococcus* showed resistant to 2 to 5 drugs.

Table 8: Multiple antibiotic resistance (MAR) indices of bacteria

MAR Index	**Frequency of MAR index**				**Total**
	E. coli	***Pseudomonas* spp. + *P. aeruginosa***	***Klebsiella* spp. + *K. pneumoniae***	***Enterococcus* spp. + *E. facealis***	
0	18	2	7	0	**27**
0.125	16	3	5	0	**24**
0.25	43	6	8	2	**59**
0.375	57	11	6	2	**76**
0.5	53	17	18	1	**89**
0.625	38	13	6	1	**58**
0.75	45	4	7	2	**58**
0.875	11	1	1	0	**13**
1	0	0	0	0	**0**
Total	**281**	**57**	**58**	**8**	**404**

4. DISCUSSION

Through this study, we aimed to determine resistance patterns in prevailing bacterial etiologic agents of UTI. The finding was then compared to prescription habits of local physicians to determine whether changes were required. This study delivers baseline information that could be utilized for defining local guidelines for the empirical treatment of UTI.

The prevalence (11.3%) of UTI in this study was found lower than all the previous recent year studies from various parts of Nepal. This figure (11.3%) was nearer to the prevalence of 14.0% and 13.9% highlighted in Dharan of Eastern Development Region and in Libya [24,25]. The prevalence rate in the various region of Nepal like Lalitpur and Kathmandu of Central Development Region and Dhangadi of Far Western Development Region are 24.08%, 27.3%, and 25.52% respectively [26-28]**.** The prevalence rate stated in eastern Nepal are lower than those stated in neighboring countries: 20.7% in Bangladesh, 33.4% in India [29,30]. This difference in prevalence could be due to differences in methodology and sample size between these latter studies and our study. Lower prevalence could also be because of peoples' tradition who feel ashamed of going for a medical checkup, self-medications, in addition to widespread private clinics and two medical college hospital nearby for which most of the patients, especially with clinical symptoms, prefer to investigate themselves.

The majority of urine samples showed no growth (87.4%) or insignificant growth (1.3%). This may be because of other clinical conditions mimicking UTI symptoms or patients undergoing antibiotics therapy which might have inhibited or destroyed the bacterial growth, or slow growing organisms which were unable to grow on the routine culture media [27,31].

In relation to gender of patients, the prevalence of UTI was significantly higher in females than in males. Female is more 63.2% more likely to develop UTI than male (OR 1.632 CI 1.306, 2.039) (Table 1). More than two-third of the incidence was seen in female (70.53%) and quite lower than one-third was in male (29.47%). These findings were in accordance with earlier studies on UTI [27]. This could be due to the anatomical differences in the female because their urethra is much shorter and closer to the anus than in males, allowing the bacteria ascend to urinary tract [32].

Regarding the distribution of UTI among patients' age groups, the highest infection (28.02%) was found among children of less than 10 years. Though there is no statistical correlation between the growth of organisms and age strata of the patients, children were followed by adults with sexually active age especially of 20-29 years showing 22.71%. The age group 10-19 years had 20.53% of UTI. From 30 years of age onwards, the UTI infection of each age group was less than 10% and decreased chronologically with the age group. More than two-third (71.26%) of UTI were seen until 29 years of age. An interesting observation was noted between incidence rate of age (till 29 years) and gender. While the male (48.28%) and female (51.72%) UTI were nearly similar in age group lesser than 10 years, UTI (female 62.35% and male 37.65%) of age group 10-19 years were roughly in agreement with our study. A higher UTI of 91.49% and 82.5% was seen in the female of age group 20-29 years and 30-39 years respectively. The findings of the group less than 10 years of age showed a deviation [33].

The main causative agent of UTI were gram negative organisms (97.3%) in our study, with *E. coli* (67.87%) followed by *Klebsiella* (14.01%) and *Pseudomonas* (13.77%). Enterobacteriaceae possess several factors for their adhesion to uroepithelium. Such bacteria colonize the uroepithelial mucosa, with pilli, fimbriae, and adhesion [34]**.** Prevalence rate of *E. coli* aligned in northwest India (67.66%), in Lalitpur (65.34%) and in Dharan (65.7%) while differed in Dhangadi (53.1%) and in Kathmandu (54%) [24,26-28,35,36]. The highest proportion of *E. coli* in UTI may be due to the variety of virulence characteristics that facilitate their intestinal carriage, persistence in the vagina and then ascent and invasion of the anatomically normal urinary tract.

The UTI share of *Pseudomonas* was similar in Dhangadi (12.24%) while *Klebsiella* differed with 21.4% [28]. Infection of *Klebsiella* was highly in agreement in northwest India (14%) [35]. Rest of the causative gram-negative bacteria were less than 1% each namely *Proteus* spp., *Citrobacter* spp. and *Acinetobacter* spp. A rise in the infection share (13.77%) of *Pseudomonas* in our studies was observed and greatly differed from other studies like 1.8% from India, 4.4% [29,30]. The result indicates a shift of causative pathogen. As for *Klebsiella,* it continues to be dominant gram negative uropathogen after *E. coli* as found in several other studies [37, 38].

A low occurrence was also seen with gram positive bacteria like *Enterococcus* (1.93%), *Staphylococcus aureus* (0.48%) and *Streptococcus* spp. (0.24%). This is contrary to other studies which reported these pathogens in much larger proportions [26-28]. Similar to our findings regarding *Enterococcus,* in northwest India reported 2% of *Enterococcus* spp [35]. These disparities in etiologic agents could be because of the different bacterial ecology in different regions, different lifestyle, hygienic conditions, availability of education, inadequate water availability and sample size.

In our study, no significant association was seen between age and type of uropathogen isolated or between sex and bacterial etiology. The result was similar to other studies [39, 40].

Multidrug resistance (MDR) was observed in 46.6% of the isolates in our study which was slightly higher than the previous report from western Nepal (42.86%), but comparatively lower than central Nepal (58.33%), eastern Nepal (86.95%) [24,26,28].

In our study 39.5% of *E. coli*, 63.16% *Pseudomonas*, 56.9% of *Klebsiella* were found to be multidrug resistant (MDR). The presence of MDR in *E. coli* was lower than the other studies conducted in various parts of Nepal like 59.1%, 48.1%, 86.95% and 90.8% and India (63%-65%) [24, 26, 28, 35]. Though the work regarding the MDR nature of *Pseudomonas* and *Klebsiella* from Nepal is few, one of the studies by Awasthi TR showed 50% of MDR among *Pseudomonas* which was slightly lower than our study [28]. On the contrary, MDR nature of *Klebsiella* by Awasthi TR reported 19.05% which was much lower than our study [28]. Such a finding indicated the threat of *Pseudomonas* and *Klebsiella* becoming MDR in high proportion. Though very few isolates namely *Proteus* (n=4) and *Staphylococcus aureus* (n=2), all were identified as MDR. 75% (n=6) of *Enterococcus* and 50% (n=1) of *Citrobacter* were MDR. *Proteus*, *Citrobacter,* and *Enterococcus* as MDR highly aligned with the finding in other study [41].

An increase in ESBL *E. coli* isolates in eastern Nepal region was seen in our study (22.8%) when compared to (17.3%), while overshadowed the figure with 38.38% [24,26]. In our study, 5.25% of *Pseudomonas* and 6.9% *Klebsiella* were reported as ESBLs. The findings of Singh and Chaudhary regarding ESBLs *Klebsiella* were greater than our study [26,42]. Since two-third of UTI were among the populations up to age 30 years, it was obvious to have ESBLs in the similar proportions. The highest ESBLs *E. coli* was found in the adult aged between 20-30 years. An alarming situation is raised with a higher ESBLs *E. coli* (21.9%) in children below 10 years of age followed by 18.75% ESBL *E. coli* among 10-20 years age group. The situation in the adult can be understood, but, the rise of ESBLs in children is threatening. As per the nature of ESBL, strains of gram negative ESBLs were completely resistant to ampicillin, cefexime, and cefotaxime. *E. coli* demonstrated high resistance to cotrimoxazole > ofloxacin > levofloxacin. *Klebsiella* and *Pseudomonas* were completely resistant to cotrimoxazole, ofloxacin, and levofloxacin. Since ESBLs were highly susceptible to amikacin and nitrofurantoin, such classes of drugs can be used for the empirical treatment.

The occurrence of a high proportion of MDR (56.9%) and rise in the emergence of ESBLs (6.9%) in *Klebsiella* spp. is of great concern since incidence rate share in UTI by *Klebsiella* is increasing by time. Resistance to drugs by *Klebsiella* may be due to the presence of the capsule, multidrug efflux pump, and greater efficiency to acquire and disseminate resistance plasmid [43].

A majority of gram negative bacteria *E. coli, Klebsiella* spp., *Pseudomonas* spp., *Proteus* spp., *Citrobacter* spp. and *Acinetobacter* spp. were highly sensitive and followed the order amikacin > nitrofurantoin > Levofloxacin > ofloxacin. Similarly, gram negative isolates were highly resistant to drugs like ampicillin, cefotaxime, and cefexime and resistivity order were ampicillin > cefotaxime > cefexime > cotrimoxazole.

The total share of gram positive isolates (n=11) was 2.66%. *Enterococcus* spp., *Staphylococcus aureus,* and *Streptococcus* were the representative isolates. While high sensitivity was shown by these gram-positive isolates towards amikacin, nitrofurantoin, levofloxacin, and cefotaxime; these isolates were highly resistant to ampicillin, cefexime, cotrimoxazole, and ofloxacin.

Resistance to class penicillin has been from the dawn of antibiotics and its use over the several decades have made the pathogen resistant to it. So, the highest resistance to ampicillin by uropathogens can be understood. The increasing resistance to cefotaxime and cefexime may be due to the increasing clinical use of third generation cephalosporins. Resistance to cotrimoxazole remains a question. It can be assumed that the use of sulfadoxine-pyrimethamine, which shares enzyme targets with cotrimoxazole, for routine malaria prophylaxis may be administered in fever without prescription.

Though ofloxacin and levofloxacin belong to the same class quinolones and have the same mechanism of action, a considerable difference in resistance was observed. A related study by Drago et al. on the comparison of activities of levofloxacin and ciprofloxacin against uropathogens showed that the resistance to the latter was generally more frequent [44]. Other studies have also supported the findings [45]. This difference has not been explained.

MARI is a tool that highlights the spread of bacterial resistance in a given population. Any MARI greater than 0.2 suggests that the strains of such bacteria originate from a location where multiple antibiotics are used or misused [23]. Taking 0.2 as a benchmark, it implies that our study has a very large proportion of the bacterial isolates (87.4%) that have been exposed to several antibiotics and thus have developed resistance to these antibiotics [17].

The antibiotic most frequently prescribed to the patients are ciprofloxacin, ofloxacin, cefexime, Cotrimoxazole, and amoxicillin/ clavulanic acid as an empirical treatment. The increased resistance to ampicillin, cefexime, and cefotaxime suggests that a change in prescription practice may be necessary. Antibiotics like levofloxacin, ofloxacin, and nitrofurantoin might be a better choice for the empirical treatment of UTI, but they have a higher cost. Since the resistance pattern of uropathogens varies considerably between regions and countries, a specific treatment recommendation may not be universally suitable. Local data on the prevalence of resistance to antibiotics is likely to be important in the empirical treatment.

5. CONCLUSION

The present study reveals an accustomed pattern with respect to genus/species of uropathogens involved in UTIs, and points towards substantial bacterial resistance to common empirically prescribed antibiotics. The high MARI in this region of Nepal highlights the need for continuous monitoring of antibiotic susceptibility profile of uropathogens. Our work suggests that newer fluoroquinolones and nitrofurantoin are better for empirical treatment of UTIs in this region. To control the increase of MDR/ESBLs, random and unauthorized distribution of the drug and haphazard disposal of hospital wastes must be checked.

LIMITATIONS OF THE STUDY

Antimicrobial susceptibility testing by dilution methods and determination of minimum inhibitory concentration (MIC) of therapeutic antibiotics would have been more concrete to support the findings of drug-resistant infections. Due to unavailability of resources, the genotype of all the ESBLs could not be detected. Investigations with multiple centers would generate even more significant ideas.

REFERENCES

[1] Worku, S., Derbie, A., Sinishaw, M. A., Adem, Y., Biadglegne, F. 2017. Prevalence of bacteriuria and antimicrobial susceptibility patterns among diabetic and nondiabetic patients attending at Debre Tabor Hospital, Northwest Ethiopia. International Journal of Microbiology, Article ID 5809494

[2] Linhares, I., Raposo, T., Rodrigues, A., Almeida, A. 2013. Frequency and antimicrobial resistance patterns of bacteria implicated in community urinary tract infections: a ten-year surveillance study (2000-2009). BMC Infectious Diseases, 13, 19.

[3] Zowawi, H.M., Harris, P.N., Roberts, M.J., Tambyah, P.A., Schembri, M.A., Pezzani, M.D., Williamson, D.A., Paterson, D.L. 2015. The emerging threat of multidrug-resistant gram-negative bacteria in urology. Nature Reviews Urology, 12 (10), 570–584.

[4] Hirsch, E.B., Zucchi, P.C., Chen, A., Raux, B.R., Kirby, J.E., Mccoy, C., Eliopoulos, G.M. 2016. Susceptibility of multidrug-resistant gram-negative urine isolates to oral antibiotics. Antimicrobial Agents and Chemotherapy, 60 (5), 3138–3140.

[5] Robin, F., Beyrouthy, R., Bonacorsi, S., Aissa, N., Bret, L., Brieu, N., Cattoir, V. 2016. ESBL-producing Enterobacteriaceae in France: inventory assessed by a multicentric study. Antimicrobial Agents and Chemotherapy, doi:10.1128/AAC.01911-16

[6] Paterson, D.L., Bonomo, R.A. 2005. Extended-spectrum beta-lactamases: a clinical update. Clinical Microbiology Reviews, 18, 657–686.

[7] Hall, B.G., Barlow, M. 2005. Revised Ambler classification of β-lactamases. Journal of Antimicrobial Chemotherapy, 55, 1050-1051.

[8] Drawz, S.M., Bonomo, R.A. 2010. Three decades of β-Lactamase inhibitors. Clinical Microbiology Reviews, 23, 160-201.

[9] Fertas-Aissani, R.E., Messai, Y., Alouache, S., Bakour, R. 2013. Virulence profiles and antibiotic susceptibility patterns of Klebsiella pneumoniae strains isolated from different clinical specimens. Pathologie Biologie, 61 (5), 209–216

[10] Vasaikar, S., Obi, L., Morobe, I., Bisi-Johnson, M. 2017. Molecular characteristics and antibiotic resistance profiles of Klebsiella isolates in Mthatha, Eastern Cape Province, South Africa. International Journal of Microbiology, Article ID 8486742.

[11] Lister, P.D., Wolter, D.J., Hanson, N.D. 2009. Antibacterial- resistant Pseudomonas aeruginosa: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clinical Microbiology Reviews, 22, 582-610

[12] Chen, F., Chen, G., Liu, Y., Jin, Y., Cheng, Z., Liu, Y., Yang, L., Jin, S., Wu, W. 2017. Pseudomonas aeruginosa oligoribonuclease contributes to tolerance to ciprofloxacin by regulating pyocin biosynthesis. Antimicrobial Agents and Chemotherapy, doi:10.1128/AAC.02256-16.

[13] Potron, A., Poirel, L., Nordmann, P. 2015. Emerging broad-spectrum resistance in Pseudomonas aeruginosa and Acinetobacter baumannii: Mechanisms and epidemiology. International Journal of Antimicrobial Agents, 45, 568-585.

[14] Poirel, L., Karim, A., Mercat, A., Thomas, I. L., Vahaboglu, H., Richard, C., Nordmann, P. 1999. Extended-spectrum beta-lactamase-producing strain of Acinetobacter baumannii isolated from a patient in France. Journal of Antimicrobial Chemotherapy, 43, 157-158.

[15] Ronald, A. 2003. The etiology of urinary tract infection: traditional and emerging pathogens. Disease-a-Month, 49 (2), 71-82.

[16] Prakash, D., Saxena, R.S. 2013. Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in Urban Community of Meerut City, India. ISRN Microbiology, Article ID 749629.

[17] Ekwealor, P.A., Ugwu, M.C., Ezeobi, I., Amalukwe, G., Ugwu, B.C., Okezie, U., Stanley, C., Esimone, C. 2016. Antimicrobial Evaluation of Bacterial Isolates from Urine Specimen of Patients with Complaints of Urinary Tract Infections in Awka, Nigeria. International Journal of Microbiology, Article ID 9740273.

[18] Cheesbrough, M. 2009. Biochemical tests to identify bacteria. In: District Laboratory Practice in Tropical Countries, Part II. 2nd ed. New York: Cambridge University Press, 45–58.

[19] Clinical and Laboratory Standards Institute. 2016. Performance standards for antimicrobial susceptibility testing: 26th informational supplement. Document M100-S26. CLSI, Wayne, USA.

[20] Kumar, D., Singh, A.K., Ali, M.R., Chander, Y. 2014. Antimicrobial Susceptibility Profile of Extended Spectrum β-Lactamase (ESBL) Producing Escherichia coli from Various Clinical Samples. Infectious Diseases: Research and Treatment, 7, 1–8.

[21] Magiorakos, A.P., Srinivasa, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G. 2011. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infectious Diseases, 18, 268-281.

[22] Rawat, D., Nair, D. 2010. Extended-spectrum β-lactamases in Gram Negative Bacteria. Journal of Global Infectious Diseases, 2 (3), 263-274.

[23] Krumpermann, P.H. 1983. Multiple Antibiotics Resistance Indexing of E. coli to Identify High Risks Sources of Faecal Contamination of Foods. Applied and Environmental Microbiology, 46 (1), 165-170.

[24] Chakrawarti, A., Dongol, P., Khanal, H., Subba, P., Banerjee, J. J. 2015. Extended spectrum beta lactamases detection and multiple antibiotic resistance indexing of Escherichia coli from urine samples of patients from a referral hospital of eastern Nepal. International Journal of Applied Sciences and Biotechnology, 3 (3), 423-426.

[25] Mohammed, M.A., Alnour, T.M.S., Shakurfo, O.M., Aburass, M.M. 2016. Prevalence and antimicrobial resistance pattern of bacterial strains isolated from patients with urinary tract infection in Messalata Central Hospital, Libya. Asian Pacific Journal of Tropical Medicine, 9 (8), 771–776.

[26] Singh, V.K., Tuladhar, R., Chaudhar, M.K. 2015. Beta lactamase producing Escherichia coli, Klebsiella pneumonia and methicillin resistant Staphylococcus aureus among uropathogens. Nepal Journal of Science and Technology, 16 (1), 105-112.

[27] Sharma, A.R., Bhatta, D.R., Shrestha, J., Banjara, M.R. 2013. Antimicrobial susceptibility pattern of Escherichia coli isolated from urinary tract infected patients attending Bir Hospital. Nepal Journal of Science and Technology, 14 (1), 177-184.

[28] Awasthi, T.R., Pant, N.D., Dahal, P.R. 2015. Prevalence of multidrug resistant bacteria in causing community acquired urinary tract infection among the patients attending outpatient department of Seti Zonal Hospital, Dhangadi, Nepal. Nepal Journal of Biotechnology, 3(1), 55-59.

[29] Yasmeen, B.H.N., Islam, S., Islam, S., Uddin, M.M., Jahan, R. 2015. Prevalence of urinary tract infection, its causative agents and antibiotic sensitivity pattern: A study in Northern International Medical College Hospital, Dhaka. Northern International Medical College Journal, 7(1).

[30] Khan, R., Saif, Q., Khan, F., Rizvi, M., Haque, F.S., Khan, S.A. 2015. Clinical and bacteriological profile of UTI patients attending a North Indian tertiary care center. Annals of Tropical Medicine and Public Health, 8 (6), 246-252.

[31] Kohanski, M.A., Dwyer, D.J., Collins, J.J. 2010. How antibiotics kill bacteria: from targets to networks. Nature Reviews Microbiology, 8, 423-435.

[32] Minardi, D., D'anzeo, G., Cantoro, D., Conti, A., Muzzonigro, G. 2011. Urinary tract infections in women: etiology and treatment options. International journal of Internal Medicine, 4, 333–343.

[33] Parajuli, N.P., Maharjan, P., Parajuli, H., Joshi, G., Paudel, D., Sayami, S., Khanal, P.R. 2017. High rates of multidrug resistance among uropathogenic Escherichia coli in children and analyses of ESBL producers from Nepal. Antimicrobial Resistance and Infection Control, 6, 9.

[34] Jacobsen, S.M., Stickler, D.J., Mobley, H.L.T., Shirtliff, M.E. 2008. Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. Clinical Microbiology Reviews, 21 (1), 26–59.

[35] Sharma, N., Gupta, A., Walia, G., Bakhshi, R. 2016. Pattern of antimicrobial resistance of Escherichia coli isolates from urinary tract infection patients: A three-year retrospective study. Journal of Applied Pharmaceutical Science, 6 (01), 062-065.

[36] John, A.S., Mboto, C.I., Agbo, B. 2016. A review on the prevalence and predisposing factors responsible for urinary tract infection among adults. European Journal of Experimental Biology, 6 (4), 7-11.

[37] Ahmed, S.M., Jakribettu, R.P., Koyakutty, S., Arya, B., Shakir, V.P.A. 2012. Urinary tract infections – an overview on the prevalence and the anti-biogram of gram negative uropathogens in a tertiary care centre in North Kerala, India. Journal of Clinical and Diagnostic Research, 6 (7), 1192-1195.

[38] Baveja, C.P., Perween, N., Aggarwal, P. 2014. Urinary tract infections in tertiary care hospital in North India: Etiology and antimicrobial susceptibility pattern. Journal of Medical Science and Clinical Research, 2 (11), 2940-2946.

[39] Nzalie, R.N., Gonsu, H.K., Koulla-Shiro, S. 2016. Bacterial etiology and antibiotic resistance profile of community-acquired urinary tract infections in a Cameroonian city. International Journal of Microbiology, Article ID 3240268.

[40] Akoachere, J.F., Yvonne, S., Akum, N.H., Seraphine, E.N. 2012. Etiologic profile and antimicrobial susceptibility of community-acquired urinary tract infection in two Cameroonian towns. BMC Research Notes, 5, 219.

[41] Shrestha, B., Gurubacharya, R.L., Maharjan, B., Shrestha, S. 2012. Multi drug resistant pathogens causing urinary tract infections in children at Kathmandu Model Hospital. Journal of Nepal Health Research Council, 32 (3).

[42] Chaudhary, P., Bhandari, D., Thapa, K., Thapa, P., Shrestha, D., Chaudhary, H.K., Shrestha, A., Parajuli, H., Gupta, B.P. 2016. Prevalence of extended spectrum beta-lactamase producing Klebsiella pneumoniae isolated from urinary tract infected patients. Journal of Nepal Health Research Council, 14 (33), 111-115.

[43] Yusha'u, M., Aliyu, H.M., Kumurya, A.S., Suleiman, K. 2010. Prevalence of Extended spectrum β-lactamases among Enterobacteriaceae in Murtala Mohammed specialist hospital, Kano, Nigeria. Bayero Journal of Pure and Applied Sciences, 3 (1), 169-172.

[44] Drago, L., Vecchi, E.D., Mombelli, B., Nicola, L., Valli, M., Gismondo, M.R. 2001. Activity of levofloxacin and ciprofloxacin against urinary pathogens. Journal of Antimicrobial Chemotherapy, 48 (1), 37–45.

[45] Jones, M.E., Visser, M.R., Klootwijk, M., Heisig, P., Verhoef, J., Schmitz, F.J. 1999. Comparative activities of clinafloxacin, grepafloxacin, levofloxacin, moxifloxacin, ofloxacin, sparfloxacin, and trovafloxacin and nonquinolones linozelid, quinupristin-dalfopristin, gentamicin, and vancomycin against clinical isolates of ciprofloxacin-resistant and susceptible Staphylococcus aureus strains. Antimicrobial Agents and Chemotherapy, 43 (2), 421–423.