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Prevalence of Esbl, Producing *Escherichia Coli* from Urine Sample in Biratnagar, Nepal

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Abstract

Background: This study was conducted in order to find out the prevalence of Extended Spectrum Beta-Lactamases producing *Escherichia coli* from the urine sample of the patients visiting Koshi Zonal Hospital Biratnagar, Nepal.

Methods: A total of 351 urine samples were processed during the study. The urine samples were cultured onto the MacConkey agar and Blood agar plates by the semi-quantitative culture technique using a standard calibrated loop have an internal diameter of 3 mm.

Results: Altogether 351 urine samples were processed during the study. From this study, the prevalence of UTI in the patient visiting Koshi Zonal Hospital was found to be 24.30%. From the urine sample of 351, a total of 82 showed significant growth. The considerable isolate was *E. coli* i.e. 63 (76.83%). The prevalence of UTI was comparatively found higher in female patients than in male patients. *E. coli* was found highly susceptible to Gentamycin (61.55%) and least from Ampicillin (1.60%) and Nalidixic acid (1.60%). A total 10 different antibiotics were tested for *E. coli*. The MAR index of antibiotic was found highest in Nalidixic acid (MARI = 0.089) and least was found in Nitrofurantoin (MARI = 0.016). Out of the entire 63 *E. coli* segregates, 61.90% were found to be multi-drug resistance (MDR) of which 28 isolates were found to be positive on ESBL screening test. Among the screen positives, 39.30 % (11/28) were confirmed as ESBL producers. The proportion of ESBL producers was significantly higher among inpatients 33.35% (3/9) than outpatients 14.80% (8/54).

Conclusions: Many isolates were found to be resistant to at least 3 - 5 antibiotics. In view of this unfold drug resistance, the practice of routine ESBL testing for uropathogenic along with conventional antibiogram would be useful for all cases which will help in the proper treatment of the patient and also prevent further development of bacterial drug resistance.

Keywords: *Urine; ESBL; Antibiotic; E. coli; UTI*

Introduction

Urinary tract infections (UTIs) are the infection of the urinary tract and is one of the most common infectious diseases. Different causative agents can be responsible for UTIs, bacteria are the major cause being responsible for more than 95% of UTI cases. In this context, *Escherichia coli* is the most prevalent organism and is solely responsible for the majority of these infections i.e., 80% UTI [1].

The newly detected plasmid-encoded resistance was selected by the frequent use of cephalosporines. Due to the presence of enzymes named Extended-Spectrum-Beta-Lactamases (ESBL) bacteria inactivate practically all cephalosporines. The phenotypes of the family *Enterobacteriaceae* which produce ESBL were primarily considered as multi resistant organisms originating in hospitals. In the recent years, an increase of such ESBL producers has been observed in outpatient settings, especially related to UTI [2]. The ESBL enzymes are plasmid-mediated enzymes and are capable of inactivating a wide variety of beta-Lactams, including third generation cephalosporins, penicillins,

and aztreonam. Plasmids responsible for ESBL production carry resistance to many antibiotics like aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and co-trimoxazole [3].

Materials and Methods

The study is carried out in Koshi Zonal Hospital from March-2015 to August-2015 to find out the prevalence of ESBL producing *E. coli* from the urine sample of the patients. The urine samples were cultured onto the MacConkey agar and Blood agar plates by the semi-quantitative culture technique using a standard calibrated loop have an internal diameter of 3 mm [4]. The protocol was followed as recommended by WHO [5]. It was then inoculated on the surface MacConkey Agar (MA) and Blood Agar (BA). The plates were then aerobically incubated for less than 37^0C overnight, the culture was reported as insignificant growth for 10^4/ml of organism, but for 10^4 - 10^5/ml organisms the culture was reported as doubtful significance (suggest repeat specimen) and for more than 10^5/ml organisms then the culture was reported as significant bacteriuria. Organisms isolated other than *E. coli* were further processed by hospital staff. The pure culture of Gram-negative bacteria was obtained on NA. Its colonial and morphological characteristics were recorded. For gram-negative bacteria catalase test and oxidase test were conducted.

It was then incubated in different biochemical media such as Triple Sugar Iron agar, SIM media, MR/VP media, Citrate media and Urease broth. Susceptibility tests of the different isolates towards various antibiotics were performed by modified Kirby-Bauer disk diffusion method for the commonly isolated pathogens using Mueller Hinton Agar (MHA). MDR isolates were screened for possible ESBL production using Ceftazidime (30 µg) and Cefotaxime (30 µg). The ESBL producers were subjected to Double Disk Approximation Assay.

Results

The study was done on 351 patients Koshi Zonal Hospital, which were belonging to all age and sex categories. Out of 351 urine samples received, 82 (23.36%) samples showed culture positive whereas the majority of samples i.e. 269 (76.64%) showed no growth (Table 1). The age group of 20 - 30 years had the maximum growth 44.50% followed by the age group 50 - 60 with 12.70% growth and age group of > 70 years had lowest with 3.30% growth (Table 2). *E. coli* was found highly susceptible to Gentamycin (61.55%), followed by Ciprofloxacin (59.95%), Nitrofurantoin (53.45%), Norfloxacin (48.60%), Cefotaxime (46.95%), Ceftazidime (40.50%), Cotrimoxazole (38.90%), ceftriaxone (32.40%) and least from Ampicillin and Nalidixic acid (1.60%) (Table 3). A total 10 different antibiotics were tested for *E. coli.* The MAR index of antibiotic was found highest in Nalidixic acid (MARI = 0.089) and least was found in Nitrofurantoin (MARI = 0.016). The MAR index > 0.2 was found in 36 (57.14%) isolates of *E. coli* (Table 4). Out of the total (351) sample processed, 63 *E. coli* isolates were found and among 63 isolates, 39 were MDR and 11 were ESBL producing (Table 5). Out of a total number of 63 isolated *E. coli*, 8 ESBL producing and 48 Non-ESBL producing *E. coli* were found in outpatient whereas 3 ESBL producing and 6 Non-ESBL producing *E. coli* were found in inpatient (Table 6). Among 11 ESBLs producers, ESBLs *E. coli* were isolated more commonly between the age groups of 20 – 30 years, were isolated in the both extremes of age (50 - 70 years) and less commonly between 10 - 20, 30 - 40, 40 - 50 and above 70 years and not isolated the age of fewer than 10 years (Table 7). Among 11 ESBL producing isolates 7 were from female and 4 were from a male. Female to male ESBL *E. coli* ratio was 1.75:1 (Table 8). In this study, all the 11 ESBLs positive *E. coli* showed resistance to Ampicillin, Ceftriaxone, Ceftazidime, Cefotaxime, Ciprofloxacin, Norfloxacin, and Cotrimoxazole and were sensitive to imipenem and Piperacillin-tazobactam. They showed variable resistance pattern to Amikacin (81.80%), Gentamicin (36.35%), Nitrofurantoin (27.20%) (Table 9). Among 82 cases of significant growth of urinary isolates, 63 (76.83%) were *E. coli* and 19 (23.17%) from others (Figure 1).

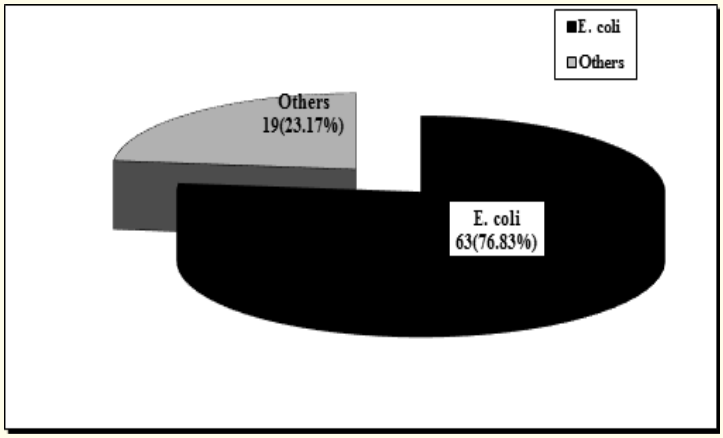

Figure 1: *Distribution of Uropathogens.*

S.N.	Growth	No. of samples	Percentage of samples
1.	Culture positive	82	23.36%
2.	No Growth	269	76.64%
Total		351	100

Table 1: *Culture result of urine samples.*

Age (years)	Male	Female	Total no. of samples	No. of *E. coli* isolates in		Isolates in percentage
				Male	Female	
<10	13	21	34	1	2	4.70
10-20	12	14	26	2	4	9.50
20-30	26	105	131	6	22	44.50
30-40	8	22	30	1	3	6.30
40-50	7	18	25	2	3	7.90
50-60	16	26	42	3	5	12.70
60-70	21	20	41	3	4	11.10
>70	12	10	22	1	1	3.30
Total	115	236	351	19	44	100
For age (P=0.567) and For gender (P=0.615)						

Table 2: *Age and Sex-wise distribution of E. coli.*

Escherichia coli (N=63)							
S.N.	Antibiotics	Resistant		Intermediate		Sensitive	
		No.	%	No.	%	No.	%
1	Nalidixic acid	56	90.30	5	8.10	1	1.60
2	Ampicillin	53	85.85	8	12.95	1	1.60
3	Gentamycin	21	34.00	3	4.85	38	61.55
4	Cotrimoxazole	37	59.95	1	1.60	24	38.90
5	Ciprofloxacin	18	29.15	7	11.35	37	59.95
6	Norfloxacin	24	38.90	8	12.95	30	48.60
7	Nitrofurantoin	10	16.20	19	30.80	33	53.45
8	Ceftazidime	23	37.30	14	22.70	25	40.50
9	Cephotaxime	21	34.00	12	19.45	29	46.95
10	Ceftriaxone	33	53.45	9	14.55	20	32.40

Table 3: *Antibiotic susceptibility pattern of E. coli.*

Escherichia coli (N=63)					
S.N.	Antibiotics	Resistant	MAR index (of antibiotics)	MAR index (of isolates)	
				≤0.2	>0.2
1	Nalidixic acid	56	0.089	27	36
2	Ampicillin	53	0.085		
3	Gentamycin	21	0.033		
4	Cotrimoxazole	37	0.059		
5	Ciprofloxacin	18	0.029		
6	Norfloxacin	24	0.038		
7	Nitrofurantoin	10	0.016		
8	Ceftazidime	23	0.037		
9	Cefotaxime	21	0.033	Total = 63	
10	Ceftriaxone	33	0.053		

Table 4: *Multiple Antibiotics Resistance (MAR) index of E. coli.*

Specimen	Total samples processed	*E. coli* isolates		MDR isolates		Suspected ESBL isolates	ESBL producing *E. coli* (%)
		No.	%	No.	%		
Urine	351	63	17.95	39	61.90	28	11 (39.30%)

Table 5: *ESBL and MDR test result of E. coli.*

No. of isolated *E. coli*	Outpatients		Inpatients	
	ESBL (%)	Non-ESBL (%)	ESBL (%)	Non-ESBL (%)
63	8 (14.80%)	46 (85.20%)	3 (33.35%)	6 (66.65%)
Total	54		9	

Table 6: *Distribution of ESBL and non-ESBL E. coli in out-and inpatients.*

Age Group	ESBL		P value
	No.	%	
<10	0	0	
10-20	1	9.10	
20-30	3	27.20	
30-40	1	9.10	
40-50	1	9.10	
50-60	2	18.20	
60-70	2	18.20	
>70	1	9.10	
Total	11	100	0.692

Table 7: *Age-wise distribution of ESBL E. coli.*

Sex	ESBL		P value
	No.	%	
Male	4	36.36	
Female	7	63.64	
Total	11	100	P= 0.801

Table 8: *Sex-wise distribution of ESBL E. coli.*

Antibiotics	Resistance		Intermediate		Sensitive	
	No.	%	No.	%	No.	%
Amikacin	0	100	2	18.20	9	81.80
Imipenem	0	00	0	00	11	100
Piperacillin-tazobactam	0	00	0	00	11	100
Ceftriaxone	11	100	0	00	0	00

Ceftazidime	11	100	0	00	0	00
Cefotaxime	11	100	0	00	0	00
Ciprofloxacin	11	100	0	00	0	00
Cotrimoxazole	11	100	0	00	0	00
Ampicillin	11	100	0	00	0	00
Gentamycin	5	45.45	2	18.20	4	36.35
Norfloxacin	11	100	0	00	0	00
Nitrofurantoin	3	27.20	5	45.45	3	27.20

Table 9: *Antibiotic sensitivity patterns of ESBL producing E. coli (n = 11).*

Discussion

This study was conducted to reveal the prevalence of ESBL producing *E. coli* from the urine sample of the patients visiting Koshi Zonal Hospital. Altogether 351 mid-stream urine samples were collected from the patients and were subjected to the standard microbiological procedure.

In this study, various population were included. Out of 351 urine specimens, 82(23.36%) showed significant growth during culture. Among them, the prevalence of UTI by *E. coli* was calculated to be 63(76.83%). In similar studies carried out by Moyo (2010) [6].

The overall prevalence of UTI (male and female) in this study was calculated to be (63/351) 17.95%, whereas the prevalence of UTI in female was calculated to be (44/63) 69.90% and in the male is (19/63) 30.10%. A similar study carried out by Ghadiri., *et al*. [7]. This result suggested that the incidence of urinary tract infection was higher in females than males. Several types of research have reported differences in the prevalence between females and males. This is due to the drier environment in the male urethra prevents the optimal growth of bacteria than that of the female. The antimicrobial activity of prostate secretions and longer distance between the anus and urethra meatus in males are among the factors responsible for the differences in the prevalence of pathogens between the two genders [8].

The age group of 20 - 30 years in this study had got the high prevalence of UTI where 28(44.50%) patients were found positive in this age group. A similar study carried out by Dias., *et al*. [9] also found the same result in their study.

In the urine isolate of *E. coli*, Nalidixic acid and Ampicillin (1.60%) were found the least susceptible. Similarly, Gentamycin (61.55%) was found most efficient antibiotics followed by Ciprofloxacin (59.95%), Nitrofurantoin (53.45%), Norfloxacin (48.60%), Cefotaxime (46.95%), Ceftazidime (40.50%), ceftriaxone (32.40%). The results found in this study is strongly supported by different other researchers. In a study conducted by Acharya., *et al*., (2011) from the urine sample of patients who attended the Chitwan Medical College, the *E. coli* isolates were found 100% resistant to ampicillin and 90.1% resistant to nalidixic acid.

MAR Index of *E. coli* isolates was calculated. Among the total 63 isolated *E. coli*, 36 showed the MAR index of bacterial pathogens higher than 0.2 which implies that strains of such bacteria originate from an environment where several antibiotics are used. The result found was strongly supported by the study conducted by Tambedkar., *et al.* (2006) [10].

A total 10 different antibiotics were tested for *E. coli*. The MAR index was found highest in Nalidixic acid (MARI = 0.089) followed by Ampicillin (MARI = 0.085), Cotrimoxazole (MARI = 0.059), Ceftriaxone (MARI = 0.053), Norfloxacin (MARI = 0.038), Ceftazidime (MARI = 0.037), Cefotaxime (MARI = 0.033), Gentamycin (MARI = 0.033), Ciprofloxacin (MARI = 0.029) and least was found in Nitrofurantoin (MARI = 0.016). This result is supported by the study conducted by Tambedkar., *et al*. [10].

Thus, present study indicates the large portion of bacteria were exposed to the antibiotics. Besides, a large number of the bacterial isolate in this study showed multiple antibiotics resistance. The present study data gives an idea about the common trend increased an-

tibiotics resistance of uropathogenic in UTI in this region, which may be due to geographic variation or indiscriminate or sub-lethal use of antibiotic.

The proportion of female and male was 1.75 : 1 in this study. Similar results showed from that of Hori., *et al.* [11] who observed female to male.

The urinary *E. coli* isolates which were ESBL producers were significantly higher among inpatients 33.35% (3/9) than outpatients 14.80% (8/54). The result found was strongly supported by the study conducted by Naik and Desai, [3].

In the present study, all the 11 ESBL producers *E. coli* were 100% sensitive to Imipenem and Piperacillin-tazobactam. Similar findings have also been reported by other workers [12].

In our study, all the isolates were resistance to Ampicillin, Ceftriaxone, Ceftazidime, Cefotaxime, Ciprofloxacin, Norfloxacin and Cotrimoxazole. They showed variable resistance pattern to Amikacin (81.80%), Gentamicin (36.35%), Nitrofurantoin (27.20%). Aminoglycosides are active against Gram-negative bacilli. Among the nonbeta-lactams, Amikacin showed good activity with 81.80%. A study was done by Ejaz., *et al*. [13] in Pakistan reported relatively lower sensitivity to amikacin (46.5%) whereas in Saudi Arabia by Al-Zahrani and Akhtar [14] reported relatively higher sensitivity to amikacin (93.7%).

Beta-lactamases continue to be the leading cause of resistance to ß-lactam antibiotics in gram-negative bacilli. In recent years, there has been an increased incidence and prevalence of ESBLs that hydrolyze and cause resistance to oxymino-cephalosporins and aztreonam [15]. For a number of reasons, the detection of ESBL-producing strains is remarkable for all major hospitals worldwide. As these strains are most likely to be even more prevalent than it is currently recognized. Due to the difficulty in their detection by the recent diagnostic methods, many of these strains have been reported to be susceptible to widely used and tested broad-spectrum ß-lactams and ESBLs constitute a serious threat to current ß-lactam therapy. Treatment of ESBL infection is difficult as the CLSI recommends that all expanded spectrum cephalosporins be taken resistant in ESBL producers. Lastly, institutional outbreaks are increasing because of selective pressure due to the heavy use of expanded-spectrum cephalosporins and also due to lapses in effective infection control measures [16].

Conclusion

From this study, the prevalence of UTI in the patient visiting Koshi Zonal Hospital was found to be 24.30%. From the urine sample of 351, a total of 82 showed significant growth. *E. coli* was the major isolates i.e. 63 (76.83%) and prevalence of UTI was comparatively found higher in female patients than in male patients.

The findings of this study showed an increase in the prevalence of resistance to a number of commonly used third-generation cephalosporins like ceftazidime, cefotaxime, and ceftriaxone, but highly responded to drugs like Imipenem and Piperacillin-tazobactam. The findings of the present study showed an increase in the prevalence of resistance to a number of commonly used antibiotics to an alarming level. Many isolates were found to be resistant to at least 3 - 5 antibiotics. For the prevention of further development of this emerging drug-resistant bacteria, the practice of routine ESBL testing for uropathogenic along with conventional antibiogram would be useful for all cases which will help in the proper treatment of the patient.

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