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Prevalence of Extended Spectrum Beta-Lactamase Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolated from Urinary Tract Infected Patients Attending Tertiary Care Hospital of Central Nepal

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ABSTRACT

Background: Urinary tract infection (UTI) is one of the major health problems in Nepal. *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) are two important bacteria associated with UTI. This study was designed to assess the prevalence of extended spectrum beta-lactamase (ESBL) producing *E. coli* and *K. pneumoniae* in urinary isolates at College of Medical Sciences and Teaching Hospital, Bharatpur, Chitwan, Nepal. **Methods:** We aseptically received 5564 mid-stream urine samples of suspected UTI patients from September 2016 to August 2018. The randomly collected 5564 urine samples were processed by standard Microbiological guidelines as recommended by Clinical and Laboratory Standards Institute (CLSI). All isolates including *E. coli* and *K. pneumoniae* were identified using the specific biochemical and sugar fermentation tests. Antibiotic sensitivity test was performed for all the isolates against all commonly used antibiotics by modified Kirby-Bauer disk diffusion method and interpreted following CLSI guidelines. First performed initial screening method then confirmed for ESBL production by phenotypic confirmatory disc diffusion test (PCDDT). **Results:** Out of 5,564 urine specimens investigated, *E. coli* was isolated in 1219 (63.99%) and *K. pneumoniae* in 223 (11.70%) cases. Initial screening revealed 615 (50.45%) isolates of *E. coli* and 127 (56.95%) *K. pneumoniae* to be resistant. Further testing by PCDDT method confirmed 102 (16.58%) *E. coli* and 25 (19.68%) *K. pneumoniae* isolates to be ESBL producers. These ESBL producers' uropathogens revealed high degree of resistance to cephalosporins (100%) and quinolones (52%-92%) group of antibiotics. **Conclusions:** In our study the prevalence of ESBL producing *K. pneumoniae* was found to be 19.68%, those of *E. coli* was to be 16.58% by PCDDT. In this study, all ESBL producing *K. pneumoniae* isolates were sensitive (100%) to meropenem and *E. coli* showed 98.04% sensitive to meropenem. Hence, for the treatment of these ESBL infections, currently, carbapenems are the recommended drug of choice.

Keywords: *E.coli;* extended spectrum beta-lactamase; *K. pneumoniae*; urinary tract infection.

INTRODUCTION

Urinary tract infections (UTIs) are one of the most common bacterial infections in humans both in the hospital and community.[1] *E. coli* and *K. pneumoniae* are the two major pathogens commonly isolated in urine.[1,2] The extended-spectrum β-lactamases (ESBLs) exist in every region of the world and in most genera of Enterobacteriaceae. ESBLs are one of the most alarming groups of β-lactamases in clinical practice. These ESBLs are major causes of both nosocomial and community-acquired infections.[3] ESBLs are either chromosomes mediated or plasmid mediated enzymes that acquired resistance by hydrolyzing penicillins, cephalosporins of the first, second, third and fourth generations (cephalexin, cefaclor, ceftazidime, cefotaxime and cefepime) and monobactams (aztreonam) but do not affect cephamycins (cefoxitin and cefotetan) or carbapenems (meropenem or imipenem) and their activity is inhibited by β-lactamase inhibitors (clavulanic acid, tazobactam and sulbactam).[4,5] TEM-1 was the first reported plasmid-mediated β-lactamase in Europe and was obtained from *Escherichia coli* in the early 1960s.[6] Afterwards ESBL was detected from *Klebsiella* in Germany 1983 and in France 1985.[7]

A therapeutic challenge for both microbiologist and clinician results when cephalosporins are not available as a treatment option. Patients with infections caused by ESBL-producing organisms tend to have less satisfactory outcomes compared to those who have infections with non ESBL-producers.[7] It has also been observed that ESBL-producing bacteria are frequently resistant to other classes of antibiotics such as quinolones, aminoglycosides, and sulfamethoxazole.[8] Therefore, it is imperative that microbiology

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laboratories should detect such infections promptly and with accuracy. Hence, this study aimed to determine the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* in urine samples of patients visiting a tertiary care hospital of Bharatpur, Chitwan, Nepal.

METHODS

A hospital based cross-sectional study was conducted in the department of Microbiology, College of Medical Sciences and Teaching Hospital (COMS-TH), a tertiary care hospital in Bharatpur, Chitwan, Nepal. The duration of study was 24 months from September 2016 to August 2018. Ethical consideration was taken from Institutional review committee (IRC) of COMS-TH. Written informed consents were obtained from patients prior to the study. For the children under five years of the age, the information was gathered from their parents. A total of 5,564 consecutive, nonrepetitive urine samples were received in the Microbiology laboratory from the OPD and hospitalized patients of different hospital units. The mid stream urine samples (10-20 ml) were collected in the sterile, clean, dry, wide mouthed leak-proof container. The idiosyncratic instruction was followed by the patient for the sample collection. The container was then labeled properly with serial number, age and sex. When instantaneous processing was not achievable, the sample was refrigerated at 4-6°C, and boric acid (1.8% w/v) was added as preservative to the urine with delay of more than 2 hours anticipated.[9]

Culture of specimens

Media were set as instructed by the manufacturer company (HiMedia). The urine specimen was streaked on the Cystine Lactose Electrolyte Deficient agar medium (CLED) with Andrad's indicator and Blood Agar (BA) medium. The semi-quantitative culture procedure *via* a standard loop method was followed on CLED medium. The culture plates were incubated aerobically at 37°C overnight. The fairly accurate numeral of colonies was count up. The number of bacteria counted by colony forming unit (CFU) per ml of urine anticipated in accordance to the volume of urine inoculated formerly and interpretated as- $\leq 10^4$ CFU/ml urine − not significant, 10^4-10^5 CFU/ml urine − doubtful significance (suggest repeat specimen) and $\geq 10^5$ CFU/ml urine − significant bacteriuria.[9-10]

Identification of the bacterial isolates

Detection and identification of significant bacterial isolates were done by using microbiological techniques as illustrated in the Bergy's manual which includes morphological appearance of the colonies, staining reactions and biochemical properties.[10]

Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar (MHA) plates by Kirby-Bauer disc diffusion method, according to Clinical Laboratory Standards Institute (CLSI) guidelines.[11] The various antibiotics used were: cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), nalidixic acid (30 µg), co-trimoxazole (25 µg), ofloxacin (5 µg), levofloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg), nitrofurantoin (100 µg), norfloxacin (10 µg), chloramphenicol (30 µg) and meropenem (10 µg).[9-10] All the antibiotic discs and the media were procured from HiMedia, Mumbai, India. Interpretation of result was found as 'Sensitive' or 'Resistant' on the basis of the diameters of zones of inhibition of bacterial growth as suggested by the disc manufacturer.[9-11] *Escherichia coli* ATCC 25922 was used as quality controls for antibiotic susceptibility testing.

Screening of potential ESBL producers

In our study, ESBL screening was performed by disk diffusion using ceftazidime (30 μg) and cefotaxime (30 μg). If the zone of inhibition (ZOI) was $\leq$ 22 mm for Ceftazidime and $\leq$ 27 mm for cefotaxime, the isolate was considered as a potential ESBL producer as recommended by CLSI.[12]

Phenotypic confirmation for ESBL producers

Phenotypic confirmatory disc diffusion test (PCDDT), as recommended by the CLSI, was performed in all isolates presumed to be ESBL producers. In this test, ceftazidime (30 μg) disks alone and in combination with clavulanic acid (ceftazidime/clavulanic acid, 30/10 μg), cefotaxime (30 μg) disks alone and in combination with clavulanic acid (cefotaxime/ clavulanic acid, 30/10 μg) disks were applied onto a plate of MHA which was inoculated with the test strain and then incubated in ambient air at 37°C for 18 hours of incubation. Those isolates which showed increase of $\geq$ 5 mm in the zone of inhibition of the combination disks in comparison to those of the ceftazidime and cefotaxime disks alone was considered as ESBL producer.[13] *Escherichia coli* ATCC 25922 (ESBL negative) and *K. pneumoniae* ATCC 700603 (ESBL positive) were used as control strains throughout the study.

RESULTS

A total of 5,564 clean catch, mid-stream urine samples from OPD and different Indoor Patients (IP) suspected of having urinary tract infection were processed for the detection of *K. pneumoniae* and *E. coli* bacteria with ESBL production. Out of 5,564 mid-stream urine samples were subjected for culture, 1905 (34.23%) samples showed significant

bacteriuria. *Escherichia coli* 1219 (63.99%) was the most common organism isolated from UTI cases followed by *K. pneumoniae* 223 (11.70%), *Candida* spp. 112 (5.88%) as shown in Table 1.

Table 1. Bacteriological profile of uropathogens isolated from UTI cases. (n=1905)

Organism isolated	Number (n)	Percentage (%)
Escherichia coli	1219	63.99
Klebsiella pneumoniae	223	11.70
Klebsiella oxytoca	16	0.84
Candida spp	112	5.88
Pseudomonas aeruginosa	102	5.35
Acinetobacter baumanii	23	1.21
Coagulase negative *Staphylococcus* (CONS)	65	3.41
Staphylococcus aureus	39	2.05
Citrobacter spp	21	1.10
Proteus spp	49	2.57
Enterobacter spp	15	0.79
Enterococcus spp	21	1.10

As indicated in Table 2, initial screening of these isolates for ESBL production showed 615 (50.45%) of *E. coli* strains and 127 (56.95%) of *K. pneumoniae*. Phenotypic confirmatory disc diffusion test (PCDDT) revealed 102 (16.58%) of *E. coli* isolates and 25 (19.68%) of *K. pneumoniae* isolates are ESBL producers. The ESBLs producers of both *E.coli* and *K. pneumoniae* strains were more in female (82.35% and 76%) than male respectively. In Table 3, ESBL positive *E. coli* (10.71%) and *K. pneumoniae* (10.53%) was common in the age group of 60 and above in female patients and 15-30 years age group in male patients both having 16.67%. The resistance of *K. pneumoniae* and *E. coli* isolates against a spectrum of 13 selected antimicrobial agents of different classes were analyzed (Table 4). ESBL isolates showed variable result in their antibiotic sensitivity pattern against commercial antibiotic discs tested. According to the susceptibility pattern, meropenem (100%, 98.04 %) was the most effective antibiotics against *K. pneumoniae* and *E. coli* followed by the

Table 2. Sex distribution of ESBLs producing isolates.

	E. coli (n=1219)			*K. pneumoniae* (n=223)		
Sex	*E.coli* isolates n(%)	Screening positive n(%)	ESBLs producer n(%)	*K. pneumoniae* isolates n(%)	Screening positive n(%)	ESBLs producers n(%)
Male	336 (27.56)	201 (32.68)	18 (17.65)	78 (34.98)	23 (18.11)	6 (24.00)
Female	883 (72.44)	414 (67.32)	84 (82.35)	145 (65.02)	104 (81.89)	19 (76.00)
Total	1219 (100)	615 (100)	102 (100)	223 (100)	127 (100)	25 (100)

Table 3. Age groups and sex distribution of patients with ESBL positive *E. coli* and ESBL positive *K. pneumoniae* isolates.

Age group (years)	ESBL positive *E. coli (n=102)*			ESBL positive *K. pneumoniae (n=25)*		
	M n(%)	F n(%)	Total n(%)	M n(%)	F n(%)	Total n(%)
<15	0	9(10.71)	9 (8.82)	1(16.67)	1(5.26)	2 (8.0)
15-30	3(16.67)	37(44.05)	40 (39.21)	1(16.67)	11(57.89)	12 (48.0)
30-45	1(5.55)	11(13.09)	12 (11.76)	0	2(10.53)	2 (8.0)
45-60	3(16.67)	18(21.43)	21 (20.59)	1(16.67)	3(15.79)	4 (16.0)
>60	11(61.11%)	9(10.71)	20 (19.61)	3 (50)	2(10.53)	5 (20.0)
Total	18(100)	84(100)	102 (100)	6(100)	19(100)	25 (100)

Table 4. Antibiotic profile of ESBL producing *K. pneumoniae isolates* and *E. coli isolates.*

SN	Name of antibiotics	ESBL producing *K. pneumoniae (n=25)*		ESBL producing *E. coli*	
		Sensitive (%)	Resistant n(%)	Sensitive n(%)	Resistant n(%)
1	Cefotaxime	0	25 (100)	0	102 (100)
2	Ceftazidime	0	25 (100)	0	102 (100)
3	Ceftriaxone	0	25 (100)	0	102 (100)
4	Nalidixic acid	2 (8)	23 (92)	25 (24.5)	77 (75.49)
5	Co-trimoxazole	10 (40)	15 (60)	19 (18.63)	83 (81.37)
6	Ofloxacin	6 (24)	19 (76)	31 (30.39)	71 (69.61)
7	Levofloxacin	12 (48)	13 (52)	44 (43.14)	58 (56.86)
8	Gentamicin	12 (48)	13 (52)	48 (47.06)	54 (52.94)
9	Amikacin	19 (76)	6 (24)	73 (71.57)	29 (28.43)
10	Nitrofurantoin	15 (60)	10 (40)	51 (50)	51 (50)
11	Norfloxacin	4 (16)	21 (84)	32 (31.37)	70 (68.63)
12	Chloramphenicol	8 (32)	17 (68)	23 (22.55)	79 (77.45)
13	Meropenem	25 (100)	0	100 (98.04)	2 (1.96)

amikacin (76%, 71.57%) and nitrofurantoin (60%, 50%) respectively.

DISCUSSION

Urinary tract infections are the second most common bacterial infections in humans, acquired both in the community and hospital settings. Extended spectrum beta lactamases are the product of the overuse of third generation cephalosporins. The ESBLs are one of the most alarming groups of beta-lactamases in clinical practice. Our study showed 1905 bacterial isolates, out of which, 1219 (63.99%) isolates were *E. coli* and 223 (11.70%) were *K. pneumoniae*. Among all bacterial isolates, *E. coli* was found to be predominant bacteria to cause UTI. Our study can be compared with similar study done by Shrestha et al reported that *E. coli* (71.3%) and *K. pneumoniae* (9.8%) isolates from UTIs in 2016.[14] Likewise, other studies also indicated that a gram negative bacterium, particularly *E. coli*, was the commonest pathogen isolated in patients with UTI.[13,15-16] This is due to the fact that *E. coli* have special virulent properties that can bind to the glycoconjugate receptor (*Gal alpha1-4 Gal*) of the uroepithelial cells of human urinary tract which can commence infectivity itself and contributory to a foremost uropathogen throughout the world and also *E. coli* is a common pathogen which is usually a commensal bacterium of human's gastrointestinal tract. The colonization rate for *K. pneumoniae* is low in healthy individuals in the general population. But, it is increased in hospitalized patients especially with long care facilities, health care manipulations like patients who are on ventilators, catheters or surgical wounds.[17] The prevalence of ESBL producers varies greatly from country to country and even among two different institutions in the same country and that continuously changes over time.[18]

The prevalence rate of ESBL producing organisms in Nepal ranges from 14.26% to 72% as reported by various studies over the past.[13-14,19-23] In our study, initial screening for ESBL production showed 56.95% *K. pneumoniae* strains. Phenotypic confirmation disk diffusion test revealed that ESBLs prevalence of *K. pneumoniae* was found to be 19.68%, which is similar to other study conducted in Nepal where 18.42% ESBL positive *K. pneumoniae* reported in 2016.[24] The prevalence of ESBL producer *K. pneumoniae* was higher than our study in studies done in Bangladesh[25] and India.[16] In our study, 50.45% of *E. coli* strains showed ESBL production by initial screening method. 16.58% of *E. coli* isolates revealed ESBL positive by PCDDT which is similar to the study in Nepal by Chander and Shrestha reported 14.26% ESBL positive *E. coli* in 2013.[13] In another studies, 7.8%, 18.5% and 25.8% ESBL producing *E. coli* reported.[20,26] In contrast to these findings, high prevalence (> 50%) of ESBL producing *E. coli* has also been reported in other studies.[27,28] In the current study, we observed that 16.58% *E. coli* and 19.68% *K. pneumoniae* isolates were ESBL producers. The high occurrence of ESBLs in *Klebsiella pneumoniae* is of great concern since infections caused by this bacterium are very common and resistance of the organism may be due to the presence of capsule that gives some level of protection to the cells, presence of multidrug resistance efflux pump and greater efficiency to acquire and disseminate resistance plasmid.[24,26]

Female had a higher rate of isolation of ESBL producing *E. coli* (82.35%) and *K. pneumoniae* (76%) which parallels the findings as reported earlier.[13] This study revealed a higher occurrence of ESBL producing uropathogen in the adult age group of 15–30 years, which is in concordance to a study done in Pakistan.[29] This study reported the antibiotic susceptibility patterns of the ESBL producing *K. pneumoniae* and *E. coli* isolates and they varied widely with the class of antimicrobial agents used. Organisms producing ESBLs are clinically relevant and remain an important cause of failure of therapy with cephalosporins. It is essential for rationalizing both prophylaxis and treatment regimens. In this study, all isolates of ESBL producing *K. pneumoniae* and *E. coli* were uniformally resistant (100%) to the third generation cephalosporin; cefotaxime, ceftazidime and ceftriaxone, which is parallel to the findings of studies done by Chander and Shrestha[13], Islam et al.[30] In our study *E. coli* showed a high resistance rate (81.37%) to co-trimoxazole whereas the *K. pneumoniae* showed resistance rate (60%) to co-trimoxazole. A comparable resistance rate of 85% and 58.33% to co-trimoxazole was shown among ESBL producing *E. coli* and *K. pneumoniae* isolates in a study conducted in Nepal[13] and Iran.[31]

A striking feature in this study was that the quinolones; ofloxacin, norfloxacin, nalidixic acid and levofloxacin demonstrated a high resistance rate varying from >55% to 76% among ESBL positive *E. coli*, while from >52% to 92% among ESBL positive *K. pneumoniae*. A lower resistant rate of norfloxacin varying from 24% to 44% had been reported in European countries.[32] This probably reflects a better management of these clinically significant infections in developed countries. Another drug of choice for ESBL producing *K. pneumoniae* and *E. coli* to nitrofurantoin were 40% and 50% resistant respectively. This result is in harmony with report from similar study conducted at national public

health laboratory, Teku, Kathmandu in 2013.[33] This study revealed resistance rates of 52% of gentamicin and 24% of amikacin by ESBL positive *K. pneumoniae* strains while, *E. coli* shown resistance rate of gentamicin 52.94% and amikacin 28.44%. In contrast, a study done in Indore, India[34] where *K. pneumoniae* showed resistance to gentamicin 69% and amikacin 38% while 59% and 33% resistance rates were shown by *E. coli*. A resistance rate of 46.7% to gentamicin was shown by *K. pneumoniae* in a report from Pakistan[35], which is similar to results of this study. This report supports that aminoglycosides have good activity against clinically important gram negative bacilli.[36] High percentages of isolates were susceptible to the carbapenems. In this study, all ESBL producing *K. pneumoniae* isolates were sensitive (100%) to meropenem and *E. coli* showed 98.04% sensitive to meropenem. The study done by Kader and Angamuthu[37] identified more than 89% of the ESBL producers were susceptible to imipenem and meropenem, whereas Mekki et al[38] found 100% isolates sensitive to the carbapenems. Our findings can also be compared with other studies done in Nepal.[13,39] Hence, use of meropenem instead of third generation cephalosporin significantly decreases the development of ESBL production in bacteria. Of all available antimicrobial agents, carbapenems are the most sensitive and reliable treatment options for infections caused by ESBL producing isolates. Therefore, restricting the use of third generation cephalosporins, along with implementation of infection control measures, are the most effective means of controlling and decreasing the spread of ESBL producing isolates.

CONCLUSIONS

Our study showed 1905 bacterial isolates, out of which 63.99% isolates were *E. coli* and 11.70% were *K. pneumoniae*. In our study the prevalence of ESBL producing *K. pneumoniae* was found to be 19.68%, those of *E. coli* was to be 16.58% by PCDDT. In this study, all ESBL producing *K. pneumoniae* isolates were sensitive (100%) to meropenem and *E. coli* showed 98.04% sensitive to meropenem. Hence, for the treatment of these ESBL infections, currently, carbapenems are the recommended drug of choice. Extended spectrum beta lactamases organisms can be identified easily by PCDDT in any peripheral laboratory of Nepal equipped with minimum resources and facilities. Therefore it is mandatory to have a regular and routine monitoring of ESBL producing clinical isolates in laboratory practice. Rational use of antibiotics (based on the laboratory detection and sensitivity) is imperative to prevent the increasing emergence of drug resistant bacteria including ESBL producing organisms.

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REFERENCES

1. Gonzalez CM, Schaeffer AJ. Treatment of urinary tract infection: what's old, what's new, and what works. World J Urol. 1999, 17(6): 372 –82.
2. Agrawal P, Ghosh A, Kumar S, Basu B, Kapil K. Prevalence of extended spectrum β lactamases among *E. coli* and *K. pneumoniae* isolates in tertiary care hospital. Indian Journal Pathology Microbiology. 2008; 51, 139-42.
3. Bradford PA. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev. 2001; 14: 933-51.
4. Bush K, Jacoby GA, Medeiros AA.A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother. 1995; 39: 1211-33.
5. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev. 2005; 18: 657- 86.
6. Datta N, Kontomichalou P. Penicillinase synthesis controlled by infectious R factors in Enterobacteriaceae. Nature. 1965; 208, 239-41.
7. Perez F, Endimiani A, Hujer KM, Bonomo RA. The continuing challenge of ESBLs.Current opinion in Pharmacology. 2007; 7(5): 459-69.
8. Hyle EP, Lipworth AD, Zaoutis TE *et al*. Risk factors for increasing multidrug resistance among extended-spectrum beta-lactamase producing *E. coli* and *Klebsiella* species. Clin Infect Dis. 2005; 40:1317-24.
9. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic Microbiology (12th Ed.). Elesvier. 2007 USA 514-618.
10. Collee JG, Miles RS, Watt B: Tests for Identification of Bacteria. In Mackie and McCartney Practical Medical Microbiology; 14th edition. Edited by Collee JG, Fraser AG, Marmion BP, Simmons A. New York: Churchill Livingstone; 1996:131–41.
11. Performance standards for antimicrobial susceptibility testing; Clinical and Laboratory Standards Institute (CLSI). M100-S25, Wayne, PA, USA, 2015.
12. Performance standards for antimicrobial disk susceptibility tests; Clinical and Laboratory Standards Institute (CLSI). M02-A11 (Approved Standard—Eleventh Edition), Wayne, Pa, USA, 2012.
13. Chander A, Shrestha CD. Prevalence of extended spectrum beta lactamase producing

Escherichia coli and *Klebsiella pneumoniae* urinary isolates in a tertiary care hospital in Kathmandu, Nepal. BMC Research Notes. 2013; 6: 487-92.
14. Shrestha A, Manandhar S, Pokharel P, Panthi P, Chaudhary KD. Prevalence of Extended Spectrum Beta-Lactamase (ESBL) producing multidrug resistance gram-negative isolates causing urinary tract infection. EC Microbiol. 2016; 4: 749-55.
15. Parajuli PN, Maharjan P, Parajuli H, Joshi G, Paudel D, et al. High rates of multidrug resistance among uropathogenic *Escherichia coli* in children and analyses of ESBL producers from Nepal. Antimicrob Resist Infect Control. 2017; 6: 1-7.
16. 16. Sasirekha B, Manasa R, Ramya P and Sneha R. Frequency and antimicrobial sensitivity pattern of extended spectrum ß-Lactamases producing *E. coli* and *K. pneumoniae* isolated in a tertiary care hospital. Al Ameen Journal Medical science. 2010; 3: 265-71.
17. 17. Yusha'u MM, Kumurya AS, and Suleiman L. Prevalence of Extended spectrum β-lactamases among Enterobacteriaceae in Murtala Mohammed specialist hospital, Kano. Journal of Pure and Applied Sciences. 2010; 3 (1): 169- 72.
18. 18. Chaudhary U, Aggarwal R. Extended spectrum β-lactamases (ESBL) - An emerging threat to clinical therapeutics. Indian Journal of Medical Microbiology. 2004; 22(2): 75-80.
19. Raut S, Gokhale S, Adhikari B. Prevalence of Extended Spectrum Beta-Lactamases among *Escherichia coli* and *Klebsiella* spp isolates in Manipal Teaching Hospital, Pokhara, Nepal. J Microbiol Infect Dis. 2015; 5(2): 69-75.
20. Pokharel RH, Thapa B, Kafle R, Shah PK and Tribuddharat C. Co-existence of beta-lactamases in clinical isolates of *Escherichia coli* from Kathmandu, Nepal. BMC Research Notes. 2014; (7): article 694.
21. Dhakal S, Manandhar S, Shrestha B, Dhakal R, Pudasaini M. Extended spectrum beta-lactamase producing multidrug resistant urinary isolates from children visiting Kathmandu Model Hospital. Nepal Med Coll J. 2012; 14 (2):136-41.
22. Poudyal S, Bhatta DR, Shakya G, et al. ESBL producing multi drug resistant clinical bacterial isolates at National Public Health Laboratory, Nepal. Nepal Med Coll J. 2011; 13(1): 3438
23. Gautam K, Pokhrel BM, Bhatta DR, Shrestha CD. Studies on extended spectrum beta lactamase (ESBL) producing *Salmonella* isolates from clinical samples of Nepal. Nepal Med Coll J. 2012; 14 (3):204-206
24. Chaudhary P, Bhandari D, Thapa K, Thapa P, Shrestha D, Chaudhary HK, et al. Prevalence of Extended Spectrum Beta-Lactamase Producing *Klebsiella Pneumoniae* Isolated From Urinary Tract Infected Patients. J Nepal Health Res Counc . 2016; 14(33): 111-5.
25. Alipourfard I, Nili NY. Antibiogram of Extended Spectrum β-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from hospital samples. Bangladesh J Med Microbiol. 2010; 4(01): 32-6.
26. Sharma J. Prospective study on Microbiology of lower respiratory tract infections and antibiotic sensitivity profile with special interest in multidrug resistant and extended spectrum β-Lactamase producing strain. M.Sc. dissertation submitted to the central department of Microbiology, Tribhuvan University, Kathmandu (2004).
27. Kaftandzhieva A, Kotevska V, Jankoska G, Trajkovska KB, Cekovska Z and Petrovska M. J Med Sci. 2009; 2(1): 36-41.
28. Shrestha S, Amatya R, Dutta R. Prevalence of extended spectrum beta lactamase (ESBL) production in Gram negative isolates from pyogenic infection in tertiary care hospital of eastern Nepal. Nepal Med Coll J. 2011; 13 (3):186-9.
29. Ullah F, Malik SA, Ahmed J. Antimicrobial susceptibility pattern and ESBL prevalence in *Klebsiella pneumoniae* from urinary tract infections in the North West of Pakistan. African Journal of Microbiology. 2009; 3(11): 670-80.
30. Islam MS, Yusuf MA, Islam MB, Jahan WA. Frequency of ESBL in surgical site infection at a tertiary care hospital. J Curr Adv Med Res. 2014; 1: 25–9.
31. Behrooozi A, Rahbar M, Yousefi JV. Frequency of extended spectrum beta-lactamase (ESBLs) producing *E. coli* and *K. pneumoniae* isolated from urine in an Iranian 1000-bed tertiary care hospital. Afr J Microbiol Res. 2010; 4(9): 881-84.
32. Vande Donk CFM, Vande Bovenkamp JHB, De Brauer EIGB, et al. Antimicrobial resistance and spread of multi drug resistant *Escherichia coli* isolates collected from nine urology services in the Euregion Meuse-Rhine. PloS One. 2012; 7(10):47707
33. Thakur S, Pokharel N, and Sharma M. Prevalence of Multidrug Resistant Enterobacteriaceae and Extended Spectrum β Lactamase Producing *Escherichia Coli* in Urinary Tract Infection. RJPBCS. 2013; 4 (2):1615-24.
34. Pathak A, Marothi Y, Kekre V, Mahadik K, Macaden R and Lundborg CS. High prevalence of extended spectrum b-lactamase producing pathogens: results of a surveillance study in two hospitals in Ujjain, India. Infect Drug Resist. 2012; 5: 65-73.

35. Abdullah FE, Mushtaq A, Irshad M, Rauf H, Afzal N, Rasheed A. Current efficacy of antibiotics against *Klebsiella* isolates from urine samples- a multi-centric experience in Karachi. Pak J Pharm Sci. 2013; 26(1):11-15.
36. Gonzalez LS, Spencer JP. Aminoglycosides: a practical review. Am Fam Physician. 1998; 58 (8): 1811-20.
37. Kader AA, Angamuthu K. Extended-spectrum beta-lactamases in urinary isolates of *Escherichia coli*, *Klebsiella pneumoniae* and other Gram-negative bacteria in a hospital in eastern Province, Saudi Arabia. Saudi Med J. 2005; 26: 956–9.
38. Mekki AH, Hassan AN, Elsayed DEM. Extended spectrum beta lactamase among multi drug resistant *E. coli* and *Klebsiella* species causing urinary tract infections in Khartoum. J Bact Res. 2010; 2:18–21.
39. Yadav KK, Adhikari N, Khadka R, Pant AD and Shah B. Multidrug resistant Enterobacteriaceae and extended spectrum β-lactamase producing *Escherichia coli*: a cross-sectional study in National Kidney Center, Nepal. BioMed Central. Antimicrobial Resistance and Infection Control. 2015; 4:42.

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