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ORIGINAL RESEARCH ARTICLE

Fecal carriage of Extended Spectrum β-Lactamases (ESBL) Producing *Escherichia coli* and *Klebsiella* spp. among School Children in Pokhara, Nepal

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Abstract

Extended-spectrum β-lactamases (ESBL) producing microbes in recent years have been a major problem in developing countries like Nepal, with limited treatment options. This study aimed to determine the prevalence of ESBL producing *E. coli* and *Klebsiella* spp. in school children in Pokhara, Nepal. The study was conducted from June to October, 2015 at the microbiology laboratory of Manipal Teaching Hospital, Pokhara, Nepal. Antibiotic Susceptibility Test (AST) was done after isolation and identification of bacterial isolates. Then, presence of ESBL enzymes in *E. coli* and *Klebsiella* spp. were tested by combination disc diffusion test using cefotaxime and ceftazidime alone and with clavulanic acid. Out of total 309 school children, 211 (68%) bacterial isolates were detected from stool samples. Among them, *E. coli* and *Klebsiella* spp. were detected in 97 (46%) and 39 (19%) stool samples respectively. Bacteria isolated from 14 (5%) stool samples were multi-drug resistant (MDR) positive. After applying combined disk method, 88 (29%) isolates were found to be ESBL producer. Emerging prevalence rate of ESBL producing *E. coli* and *Klebsiella* spp. are major problem in medical history. Therefore, rapid need of surveillance for effective management of such MDR-strain is required.

Keywords: Extended-spectrum β-lactamase (ESBL), *E. coli*, *Klebsiella* spp., children, Nepal

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Introduction

Extended-spectrum β-lactamases (ESBL) producing organism was first isolated in 1983, Germany, since it has been increasingly reported worldwide [1, 2]. The prevalence of ESBL-positive isolates depends upon wide range of environmental, genetic, geographical, age group, and type of infections [3]. Emerging and increasing incidence of ESBL related infections has been observed throughout the world [4]. ESBLs are Class A β-lactamases carbapenems and are often plasmid-mediated enzymes with various genotypes [1]. The most common genotypes are the SHV, TEM, and CTX-M types [5]. Other clinically important types include VEB, PER, BEL-1, BES-1, SFO-1, TLA, and IBC [6]. *Enterobacteriaceae* family of gram-negative organisms; in particular, *K. pneumoniae, K. oxytoca* and *Escherichia coli* produce ESBLs [7]. They are also produced by other gram-negative organisms, such as *Acinetobacter baumannii, Proteus* spp., *Pseudomonas aeruginosa,* and *Salmonella* spp. [8].

Patients suffering from ESBL producers have high mortality, longer hospital stay, high health care cost, and longer antibiotic therapy as compared to those patients suffering from non-ESBL producers [9]. Furthermore, they pose significant therapeutic challenges in the daily management of infectious diseases due to their resistance to additional classes of antibiotics reducing the effectiveness of alternative antimicrobial regimens [10]. Worldwide, several studies [1, 5, 6, 8] have been conducted but only a few data are available concerning the genetic characterization of clinical isolates from Nepal [7, 11]. The acquisition and expression of ESBL enzymes among *E. coli* and *Klebsiella* spp. have posed a serious public health problem. This study was conducted to determine the ESBL producing bacteria in school going children without any clinical symptoms to observe the fecal carriage.

Study designing and setting

The study was conducted in Pokhara Lekhanath Municipality-26, where samples were collected

from three government (*n*=147) and three private schools (*n*=176). Children from 4 to 15 years were enrolled who were apparently healthy and not taking any antibiotics 1 week prior to our study. After taking written informed consent and questionnaire from their parents, stool samples were collected in a sterile screw-capped container. Demographic data like age, sex, antibiotic intake within 15 days, were included in questionnaire form. A total of 323 children were enrolled, where all children provided stool samples, except 14 who provided insufficient stool samples without any labeling.

Sample collection and Processing

Around one gram of fresh stool sample was collected in a clean, well labeled, and sterile screw-capped plastic container. Collected samples were kept in an icebox, transported within 1 hour, and processed immediately following Standard Operating Procedures (SOPs) of Microbiology to the laboratory of Manipal Teaching Hospital (MTH) in Pokhara, Nepal. If there was a delay in processing the specimens were kept in a freezer at 2–8°C. The general procedure of sample processing were explained in the flowchart (**Figure 1**).

Isolation and identification of *E. coli* and *Klebsiella* spp.by culture method

Stool samples were inoculated onto MacConkey agar plates, which were incubated at 37°C for 24 hours. After proper incubation, plates were observed for the growth of the bacteria. Isolated colonies were sub-cultured in nutrient agar and then plates were incubated at 37°C for 24 hours. Gram-positive and negative bacteria were initially confirmed by gram staining and thereafter, identified by conventional biochemical tests such as (MRVP, Indole, motility, protease, citrate, & O/F).

Antimicrobial Susceptibility Testing (AST)

AST of all isolates was performed by Kirby Bauer's disc diffusion method and interpretation of the results was done as described in Clinical Laboratory Standard Institute (CLSI) guideline 2016 [18]. For antibiotic susceptibility testing, antibiotic discs (HiMedia Laboratories Pvt. Ltd., India) cefotaxime (30µg), ceftazidime (30µg), gentamycin (10µg), imipenem (10µg), nitrofurantoin (300µg), norfloxacin (10µg), piperacillin-tazobactam (100µg/10µg), and amikacin (30µg) were used. Control strains of *E. coli* ATCC 25922 were used in parallel as a part of quality control. Organisms showing resistance towards two or more classes of antimicrobial agents were considered to be multidrug-resistant (MDR).

Screening and Confirmation of ESBL producing Strains

All the *E. coli* isolates were subjected to the screening test for ESBL detection. Screening test for ESBL detection was done according to the CLSI guidelines. Isolates showing inhibition zone size of ≤ 22 mm with ceftazidime (30 µg), ≤ 25 mm with ceftriaxone (30 µg), and ≤ 27 mm with cefotaxime (30 µg) were interpreted as screening test positive for ESBL production [18].

For the confirmatory test for ESBL, two or three colonies of organisms were suspended in 0.5 ml of sterile broth and the turbidity matched to 0.5 McFarland. Using a sterile cotton swab the broth culture was uniformly swabbed on MHA. All the *E. coli* isolates which were resistant to at least ceftazidime, ceftriaxone and/or cefotaxime were subjected to the ESBL confirmatory test using ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 µg & 10 µg) and the cefotaxime (30 µg) and cefotaxime-clavulanic acid (30 µg & 10 µg) combination disks. The tests were interpreted and a difference of 5 mm between zone of inhibition of a single disk and in combination with clavulanic acid (inhibitor) was confirmed to be produced by an ESBL positive isolate [18]. *Klebsiella pneumoniae* ATCC 700603 was used as a control strain.

Statistical analysis

Pearson's Chi-square test was used to determine the significant association of dependable variables. WinPepi software (version 11.65) was used for quantitative data analysis. A p-value < 0.05 was considered to be statistically significant at 95 % CI.

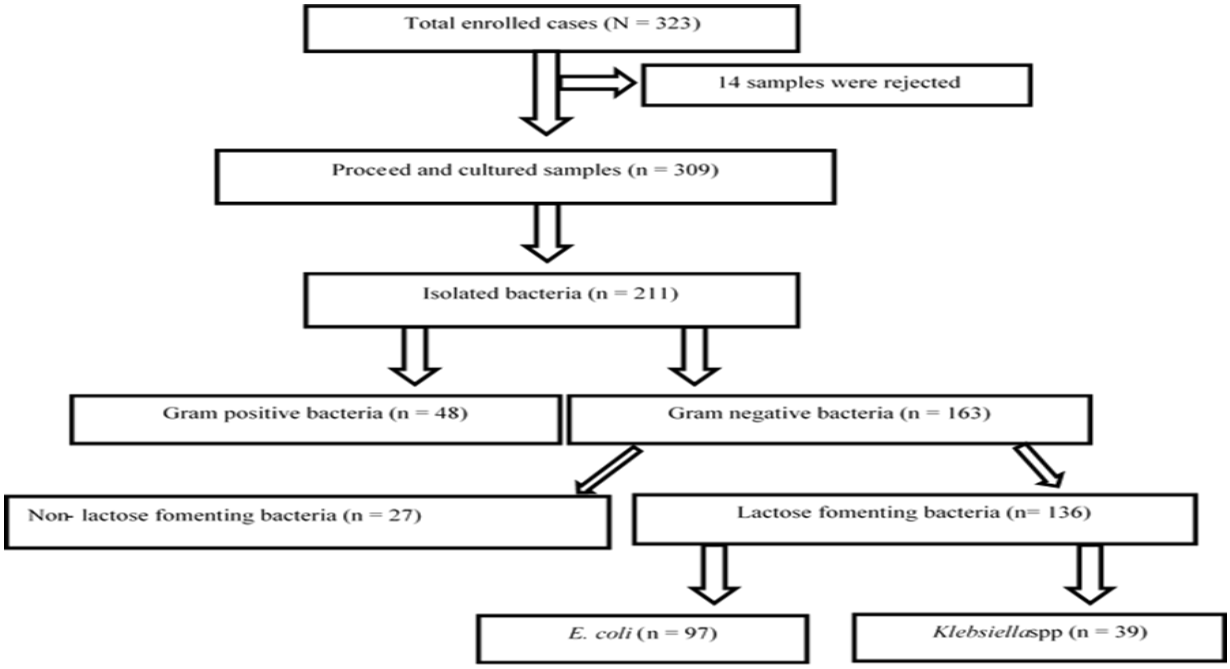

Figure 1: Flowchart of isolation and identification of *E. coli* and *Klebsiella* spp. from collected stool samples

Table 1.**Age and sex wise distributions in *E. coli* and *Klebsiella* spp. in school children**

Gender	Female			Male		
Age (year)	**Total**	***E. coli***	***Klebsiella* spp.**	**Total**	***E. coli***	***Klebsiella* spp.**
4-5	7	2 (29)	0 (0)	6	3 (50)	0 (0)
6-10	104	32 (31)	15 (14)	127	41 (32)	19 (46)
11-15	32	9 (28)	3 (9)	33	10 (30)	2 (20)
Total	**143**	**43 (30)**	**18 (16)**	**166**	**54 (33)**	**21 (13)**

Table 2. Distribution of *E. coli* and *Klebsiella* spp. on the basis of ESBL producer and MDR

Bacterial Isolates	Total no. isolates (%) (n =211)	Multidrug resistance (MDR) isolates number (%) (n =14)	ESBL screening positive (%) (n =116)	ESBL producer confirm (%) (n =88)
E. coli	97 (46)	9 (64)	84 (72)	74 (84)
Klebsiella spp.	39 (19)	5 (36)	32 (10)	14 (16)
Others gram-negative bacteria	27 (13)	NT	NT	NT
Gram-positive bacteria	48 (23)	NT	NT	NT

Results

As summarized in **Table 1**, in 143 females, *E. coli* and *Klebsiella* spp. were isolated from 43 (30%) and 18 (16%) respectively. In 166 male children, *E. coli* and *Klebsiella* spp. were isolated from 54 (33%) and 21 (13%) respectively. *E. coli* and *Klebsiella* spp. were most frequently found in children aged 6-10 years both in females (31% and 32%) and males (14% and 46%), respectively.

Of the total samples tested, 211 (68.3%) bacterial isolates were detected. Among them, *E. coli* and *Klebsiella* spp. were detected in 97 (46%) and 39 (19%) stool samples respectively. Of the total tested samples, 14 (16%) isolates of bacteria were

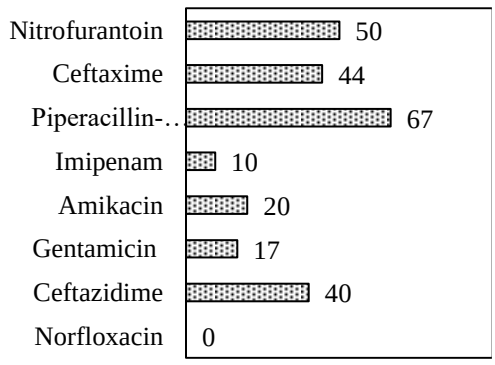

Figure 2. Antibiotic resistance pattern of *Klebsiella* spp.

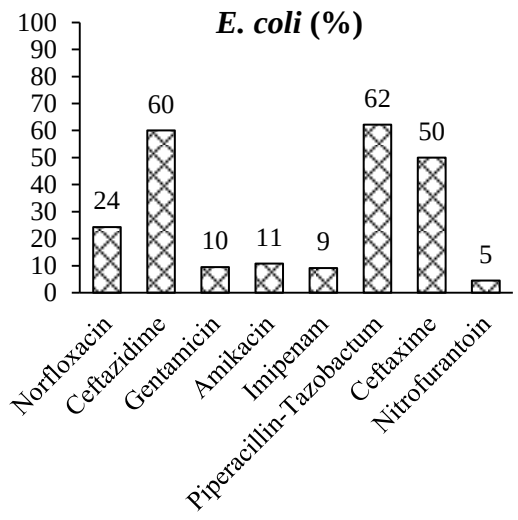

Figure 3: Antibiotic resistance pattern of *E. coli.*

Figure 2 summarizes that *Klebsiella* spp. was resistance to piperacillin-tazobactam (67%); nitrofurantoin (50%) and cefotaxime (44%). As depicted in **Figure 3**, the resistance pattern of *E. coli* was also found highest in piperacillin-*tazobactam* (62%) followed by norfloxacin (60%) and cefotaxime (50%) etc. found MDR strains, while 116 isolates were screened as ESBL positive. By combined disk method, 88 isolates were confirmed to be ESBL producer. Among the total MDR bacteria, 9 (64%) were *E. coli* and 5 (36%) were *Klebsiella* spp., 74 (84%) isolates of *E. coli* and 14 (16%) of *Klebsiella* spp. were confirmed as ESBL producer (**Table 2**).

Discussion

ESBL producing gram-negative bacilli, particularly *K. pneumoniae* and *E.coli*, recently arose worldwide in a hospital and Community-Acquired Infections (CAIs) as serious pathogens, and has been progressively rising. This upsurge introduces additional difficulties in treating patients affected by EBSLs since it puts at risk the activity of numerous wide-spectrum antibiotics [7]. The present study exhibits that out of the 211 clinical isolates, 88 (42%) were found to be ESBL producers, and these results were in accordance with a previously conducted study [11, 18]. Additionally, the ratio of positive cases for the male to female children in this study was 1.2:1, which contrasted another study where the ratio for male to female patients was 1:2.2 [7]. However, this difference in the ratio may be due to a gender bias of male and female samples initially in both the experiments. Thus, gender may not play a role in ESBL susceptibility of a person. Furthermore, *E. coli* positive cases were found in 97 (31%) clinical isolates, which was the predominant organism in this case. Conversely, another study found *E. coli* to be positive in 81% of their samples, which is remarkably higher than that of our study [7]. The high occurrence of positive result may suggest that there is a possibility of normal flora of human *E. coli.*

Anti-microbial Resistance (AMR) is a major public health problem in patient care in both developed and developing countries. Therefore, in this study, we analyzed eight different antibiotics. Among eight antibiotics, *Piperacillin-Tazobactam* resistant was predominant in both *E. coli* (62%) and *K. pneumoniae* (67%), which differed from previous studies [13–15]. *Piperacillin-Tazobactam* is commonly used to treat intra-abdominal, lower respiratory, urinary tract, and gynecological and skin/soft tissue infections, as well as for fever in patients with neutropenia [16].

The predominance of the resistant bacteria for these antibiotics could imply that illnesses associated to *E. coli* & *K.* spp. are fairly prevalent in the Pokhara and have been treated just as normally. In addition, the rates of resistance to cefotaxime, ceftazidime, gentamycin, and amikacin for *E. coli* presented in this study are 50%, 60%, 10%, and 11% respectively. However, this contrasts another study, where the resistance of *E. coli* were found 99%, 78%, 29% and 4% to cefotaxime, ceftazidime, gentamycin, and amikacin respectively [17]. This may suggest that the *E. coli* collected from Nepal has not been exposed to the four antibiotics quite as much as in other more developed places, which could probably mean that it is still possible to more easily manage the ESBLs in Nepal effectively

compared to places with higher antibiotic exposure and resistance. From the eight antibiotics that were analyzed, Imipenem was the most effective drug; however, it should not be administered as the empirical drug unless the infection is life-threatening, since carbapenems are considered the drug of last resort. If this situation is not taken seriously and misuse the carbapenem drugs then carbapenem resistant bacteria may evolve.

In this study, 4.5% MDR strains were detected, which was very low compared with other studies 64.0%, and 64.9% [19]. This may be the case due to the overexposure of the antibiotics to the bacteria in external environments and animals, whereas in the related district it may not be the case.

Furthermore, 84% *E. coli* was confirmed as ESBL producer followed by *K. pneumoniae*, (9%) and *K. oxytoca* (7%). A similar result was found in the study where the researchers reported 91% *E. coli* and 8% *K. pneumonia* [7]. Conversely, the findings of another study [20] showed the variable result with *E. coli* isolates, 33(9%) were ESBL producers. The identification of ESBL producing organisms is a major challenge in the clinical world and, due to the selective pressure caused by heavy use of expanded-spectrum cephalosporins, lapses in effective infection control measures and affinity of these enzymes for different substrates, outbreaks are increasing [21].

An antibacterial choice is often complicated by MDR leading to over-prescription and misuse of antibiotics. As indicated by the present and previous findings, it appears to be mandatory to include ESBL detection in routine laboratory practice so as to limit the rapid spread of ESBL producing organisms. AMR can be tackled by following continuously the 5 steps viz. awareness; surveillance; stewardship; research & innovation; infection prevention & control accordance with the "One Health" concept in agriculture, veterinary, environment and human.

Limitation of study:

We couldn't perform genotyping test and MBL test in bacterial isolates due to a limited resource and time limitation.

Conclusions:

The prevalence of ESBL producing *E. coli* and *Klebsiella* spp. was more. The preponderance of ESBL producing *E. coli* and *Klebsiella* spp. were resistant to the in-use antibiotics used from stool samples. Imipenem was the most potent antibiotic and could be the drug of choice for treatment of infections caused by ESBL strains. This clinical threat of raised up ESBL prevalence is creating significant therapeutic problems prompting an immediate need to formulate strategic policy initiatives to reduce their prevalence.

Conflict of Interest

The authors declare that they have no competing interests.

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Consent for Publication

Not applicable

Consent to Participant

Written informed consent was taken from all participating patients or from guardian on the behalf of their children.

Ethical approval and consent to the participant

No patient related data were collected. Ethical approval was therefore not required. The study was laboratory-based basic science study. Written informed consent was taken from all participants or from guardian on the behalf of their children.

Availability of data and Materials

All supplementary files, data generated and analyzed during this study will be made

available as per reasonable request to the corresponding author.

Source of Support

No funding was obtained.

Author's Contributions

BPB and BKS designed the study and collected sample at Manipal Teaching Hospital. DRB and BPB performed investigation and recorded the laboratory findings with the validation. HSS supervised and provided a methodology for the study. BPB, BB and RRG administered the project, reviewed literature, and wrote the original manuscript. BB and RRG curated data to performed statistical data analysis and data interpretation. RRG & BB also reviewed, proofread, and revision of the draft by compiling, formatting, editing and writing the final version of the article. Thus, all authors made a substantial contribution to the study. All of them read and approved the final manuscript.

References

1. Thenmozhi S, Moorthy K, Sureshkumar BT, Suresh M: **Antibiotic Resistance Mechanism of ESBL Producing Enterobacteriaceae in Clinical Field: A Review**. *Int J Pure Appl Biosci*. 2014, **2:**207–226. doi:10.5935/1678-9741.20110056.
2. Rupp ME, Fey PD: **Extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae: considerations for diagnosis, prevention and drug treatment**. *Drugs*. 2003, **63**:353–365. http://dx.doi.org/10.2165 /00003495-200363040-00002.
3. Livermore DM, Canton R, Gniadkowski M, et al: **CTX-M: changing the face of ESBLs in Europe.** *J Antimicrob Chemother*. 2007,**59**(2):165-174. [http://dx.doi.org/10.1093/jac/dkl483] [PMID: 17158117]
4. Arnold, RS, Thom, KA, Sharma, S, Phillips, M, Kristie Johnson, J, & Morgan, DJ: **Emergence of Klebsiella pneumoniae carbapenemase-producing bacteria** *Southern medical journal*. 2011, **104**(1): 40-45.
5. Kaur M & Aggarwal A: **Occurrence of the CTX-M, SHV and the TEM genes among the extended spectrum β-lactamase producing isolates of enterobacteriaceae in a tertiary care hospital of north India.** *J Clin Diagnostic Res*. 2013, **7**:642–645. doi:10.7860/JCDR/2013/5081.2872.
6. Akinci E & Vahaboglu H: **Minor extended-spectrum -lactamases, Expert Rev.** *Anti Infect Ther***.** 2010, **8**: 1251–1258. doi:10.1586/eri.10.119.
7. Shakya P, Shrestha D, Maharjan E, Sharma VK, & Paudyal R: **ESBL Production Among and spp. Causing Urinary Tract Infection: A Hospital Based Study.** *Open Microbiol J*. 2017, **11**: 23–30. doi:10.2174/1874285801711010023.
8. Bialvaei AZ, Kafil HS, Leylabadlo HE, Asgharzadeh M, & Aghazadeh M: **Dissemination of carbapenemases producing gram-negative bacteria in the middle-east, Iran.** *J Microbiol*. 2015, **7:** 226–246.
9. Benner KW, Prabhakaran P, & Lowros AS: **Epidemiology of Infections Due to Extended-Spectrum Beta-Lactamase–Producing Bacteria in a Pediatric Intensive Care Unit**. *J Pediatr Pharmacol Ther*. 2014, **19**: 83–90. doi:10.5863/1551-6776-19.2.83.
10. World Health Organization: **WHO Global Strategy for Containment of Antimicrobial Strategy for Containment of Antimicrobial Resistance.** 2001, **99** doi:WHO/CDS/CSR/DRS/2001.2 99 .
11. Parajuli NP, Maharjan P, Joshi G, & Khanal PR: **Emerging Perils of Extended Spectrum β -Lactamase Producing Enterobacteriaceae Clinical Isolates in a Teaching Hospital of Nepal.** *Biomed Res Int*. 2016 : 1155 doi:10.1155/2016/1782835.
12. Clinical and Laboratory Standards Institute: **Performance Standards for Antimicrobial Susceptibility Testing: Twenty-sixth informational supplement.** CLSI document M100-S26. CLSI 2016, PA: In. Wayne, USA.
13. Somily AM, Habib HA, Absar MM, et al: **ESBL-producing *Escherichia coli* and *Klebsiella pneumonia* at a tertiary care hospital in Saudi Arabia**. *J Infect Dev Ctries*. 2014, **8**(9): 1129-1136. [http://dx.doi.org/10.3855/jidc.4292] [PMID: 25212077]
14. Chander A, & Shrestha CD: **Prevalence of extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumonia* urinary isolates in a tertiary care hospital in Kathmandu, Nepal.** *BMC Res Notes*. 2013, **6**: 487. [http://dx.doi.org/10.1186/1756-0500-6-487] [PMID: 24274894]
15. Mahdi Yahya Mohsen S, Hamzah HA, Muhammad Imad Al-Deen M, & Baharudin R: **Antimicrobial susceptibility of *klebsiella pneumoniae* and *Escherichia coli* with extended-spectrum β-lactamase associated genes in hospital**

TengkuAmpuanAfzan, Kuantan, Pahang. *Malays J Med Sci*. 2016, **23**(2): 14-20. [PMID: 27547110]

16. Perry M C & Markham A: **Piperacillin/tazobactam: an updated review of its use in the treatment of bacterial infections.** *Drugs*. 1999,**57**: 805-843.
17. Michael P, Christoph E, Constanza W, Martin H, Linda F, Angelika F, et al: **Molecular characterization of extended-spectrum b-lactamase (ESBL)-producing Escherichia coli isolates from hospital and ambulatory patients in Germany**. *Veterinary Microbiology*. 2017, **200**: 130–137.
18. Baral P, Neupane S, Marasini BP, Ghimire KR, Lekhak B, & Shrestha B: **High prevalence of multidrug resistance in bacterial uropathogens from Kathmandu, Nepal.** *BMC Res Notes*. 2012, **5** :38. doi:10.1186/1756-0500-5-38,
19. Thakur S, Pokhrel N, & Sharma M: **Prevalence of multidrug resistant Enterobacteriaceae and extended spectrum β lactamase producing *Escherichia coli* in urinary tract infection.** *Res J Pharm BiolChemSci*. 2013, **4**(4): 1615-1624.
20. Parajuli NP, Maharjan P, Parajuli H, 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): 9.[http://dx.doi.org/10.1186/s13756-016-0168-6] [PMID: 28096977
21. Ullah F, Malik SA, & Ahmed J: **Antibiotic susceptibility pattern and ESBL prevalence in nosocomial *Escherichia coli* from urinary tract infections in Pakistan.** *Afr J Biotechnol*. 2009, **8**(16): 3921-3926]