

Original Article**Study of Extended Spectrum Beta-Lactamases Producing *Escherichia coli* and *Klebsiella* species in a Tertiary Care Hospital, Biratnagar, Nepal**

Kumari Ragani Yadav*, Ganesh Kumar Singh, Sujit Bhattacharjee and Kewal Shrestha

Department of Microbiology, Nobel Medical College Teaching Hospital Biratnagar, Nepal

Article Received: 25th July, 2019; Accepted: 24th November, 2019; Published: 31st December, 2019DOI: <http://dx.doi.org/10.3126/jonmc.v8i2.26743>**Abstract****Background**

Escherichia coli and *Klebsiella* species are most common ESBL producers and associated with UTI in both community and hospital setting, often limiting the treatment therapy of UTI. Aim of present study was Isolation and Identification of ESBL producing *E. coli* and *Klebsiella* Species in urine sample from cases of UTI and their antibiotic susceptibility pattern.

Materials and Methods

This descriptive cross-sectional study was conducted at Nobel Medical College Teaching Hospital with effect from October 2018 to June 2019 after approval from the Institutional Review Committee (IRC) of the college as per clinical laboratory standards institute of (CLSI) guidelines.

Results

Out of total 2567 urine samples, 631(24.5%) samples showed microbial growth. Among which *E. coli* was 288 (45.6%), *Klebsiella* species were 20(31.5%), other gram-negative bacteria were 158(25%), gram positive bacteria were 114(18%) and fungus 51 (8%) were recovered. Out of 631 culture positive urine samples, 308 urine samples were positive for *E. coli*/*K. species* 288 (93.5%)/20 (42.5%) respectively. Among which ESBL isolates were 213 (69%) and non ESBL isolates were 95 (31%). All ESBL producing *E. coli* and *K. species* were found (100%) sensitive to meropenem. Similarly, among other antibiotics also showed increased susceptibility towards the piperacillin/tazobactam (97.2%), cefoperazone/sulbactam (94.4%) and amikacin (93.0%).

Conclusion

The highest numbers of ESBL producers *E. coli* and *K. species* from urine sample are increasing day by day and creating serious problem in treating patients in Nepal. So it should be mandatory and very essential to have routine monitoring system to detect the ESBL producing isolates in clinical laboratories.

Keyword: Antibiotic, *Escherichia coli*, Urinary Tract Infections

Licensed under CC BY 4.0 International License which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

***Corresponding author**

Dr. Kumari Ragani Yadav

Lecturer

Email: raginiy385@gmail.comORCID: <https://orcid.org/0000-0003-0038-473X>**Citation**

Yadav KR, Singh GK, Bhattacharjee S, Shrestha K, Study of Extended Spectrum Beta-Lactamases Producing *Escherichia coli* and *Klebsiella* Species in a Tertiary Care Hospital, Biratnagar, Nepal, JoNMC. 8:2 (2019) 31-36.



Introduction

The organisms producing Extended Spectrum Beta- Lactamases (ESBL) enzymes are resistant to all penicillins and 1st, 2nd and 3rd generation cephalosporins and monobactam, however remains sensitive to carbapenems and cephamycin [1]. ESBL are commonly detected in the members of the Enterobacteriaceae like *Klebsiella pneumoniae* and *E. coli*. *E. coli* and *Klebsiella pneumoniae* are most two predominant and important bacteria isolated from urine samples in cases of UTI in both in hospital setting and community [2].

The multidrug resistant gram negative bacteria are serious global problem at present [3]. The easy transmission of the ESBL coding plasmids between the species has become a major threat in hospital setting and outbreaks occurring in hospital are often due to infection caused by organisms producing ESBL [4]. This issue is of great challenge and inviting first priority to every laboratory to detect ESBL isolates.

Aim of Present study was Isolation and Identification of ESBL producing *E. coli* and *Klebsiella* species in urine sample from cases of UTI and their antibiotic susceptibility pattern in Nobel Medical College Teaching Hospital, Biratnagar, Nepal.

Materials and Methods

This descriptive cross-sectional study was conducted at Nobel Medical College Teaching Hospital, Biratnagar, Nepal with effect from October 2018 to June 2019 after approval from the Institutional Review Committee (IRC) of the college. The studies consider 95% confidence interval and 80% power to evaluate the sample size. According to literature review the rate of ESBL producing *E. coli* and *Klebsiella* species in urine sample from cases of UTI is estimated approximately 50%. So by using formula $n = Z^2 pq / l^2$ where $Z = 1.96$ at 95% confidence interval, $p = 50\%$, $q = 50\%$ and $l = 20\%$ of p.i.e. 10. So now $n = 4 \times 50 \times 50 / 100 = 100$. But load of patient attending to the microbiology laboratory was high so total 2567 cases of UTI included during the study. Study populations were cases of urinary tract infection patients attending to Hospital. Inclusion criteria for this study was all the age groups from cases of UTI and the samples that were received unlabeled, collecting tubes and bottles which were cracked or broken and urine sample from non- UTI cases was excluded. Data collection was done by filling self-structured Proforma designed and endorsed by the department for the study. Data was collected throughout the study to meet the sample size for the study. A total 2567 clean catch mid-stream urine (MSU) samples

was collected in wide mouth sterile container from cases of UTI attending in hospital. All the mid-stream urine samples were inoculated on cysteine lactose electrolyte-deficient agar (CLED) and culture plates were incubated into the incubator at 37°C for overnight. The colonies from significant bacterial growth plate were processed and organisms were identified by standard microbiological techniques by using various biochemical tests and other confirmatory tests [5]. Antibiotic susceptibility testing was performed by Kirby-Bauer's disc diffusion method as per CLSI guidelines [6]. Different antimicrobial disc used were: nitrofurantoin (NIT) 300µg, norfloxacin (NX) 10µg, gentamycin (GEN) 10µg, levofloxacin (LE) 5µg, ampicillin (AMP) 10µg, cefuroxime (CXM) 30µg, ceftazidime (CAZ) 30µg, cefotaxime (CTX) 30µg, meropenem (MRP) 10µg, piperacillin /tazobactam (PTZ)100/10µg, ampicillin/salbactam (A/S) 10/10µg, amikacin (AK) 30µg, co-trimazole (COT) 25µg, ciprofloxacin (CIP) 5µg and cefoperazone /salbctum (CFS) 5/30µg. According to CLSI guidelines, [7] the organisms (*Escherichia coli* and *Klebsiella* species) were screened for ESBL production using ceftazidime (30µg), cefotaxime (30µg) and ceftriaxone (30µg). The *Escherichia coli* and *Klebsiella* species showing reduced susceptibility to at least one of these antibiotic with zone of inhibition on Mueller Hinton Agar for ceftazidime (30µg) $\leq 22\text{mm}$, $\leq 25\text{mm}$ with ceftriaxone (30µg) and $\leq 27\text{mm}$ with cefotaxime (30µg) were considered as potential ESBL producing organisms and selected for phenotypic confirmatory test for ESBL.

Phenotypic confirmatory test

The antibiotic susceptibility test were performed on Mueller Hinton Agar by using disc of third generation cephalosporins alone and disc of third generation cephalosporins along with clavulanic acid by Kirby-Bauer's disc diffusion method following the CLSI guidelines [6]. The suspected ESBL producing *Escherichia coli* and *Klebsiella* species were inoculated as lawn culture on Mueller Hinton Agar. Then the discs of ceftazidime (30µg) alone and ceftazidime + clavulanic acid (30µg +10µg) discs along with cefotaxime (30µg) discs alone and cefotaxime + clavulanic acid (30µg +10µg) discs were placed with 25 mm apart on lawn culture of organism on Mueller Hinton Agar. After overnight of aerobic incubation into the incubator at 37°C the difference in zone diameter of cephalosporin alone and cephalosporins + clavulanic acid were measured by using verniercaliper scale. An increase of ≥ 5 mm of zone diameter around cephalosporins + clavulanic acid disc compared to cephalosporins alone was confirmed as ESBL producer.



Antibiotic susceptibility pattern of isolated ESBL

ESBL producer isolates were further tested against different antibiotics: levofloxacin (5µg), meropenem(10µg), piperacillin/tazobactam(100/10µg), ampicillin/salbactm(10/10µg), amikacin (30µg), co-trimazole (25µg), ciprofloxacin (5µg) and cefoperazone/salbctum(5/30 µg) on MHA by Kirby-Baur's disc diffusion method as per CLSI guideline [6].

Statistical analysis

The collected data were analysed using statistical package for the social science for windows (SPSS) version 20. Parametric variables were assessed using chi-squared test, as appropriate. A difference was considered statistically significant if the p-value <0.05.

Results

Out of total 2567 urine samples, 631 (24.5%) samples showed microbial growth. Among which 288 (45.6%), 20 (31.5%), 158 (25.0%), 114 (18.0%) and 51(8.0%) samples showed *E. coli*, *Klebsiella* species, other gram negative bacteria, gram positive bacteria and fungus were recovered respectively (Table1). The cases of UTI which is caused by *E. coli* and *K. species* were higher in female 246 (80.0%) as compared to male 62 (20.0%). Male: female ratio was 1:3.9. This difference is statistically significant with p value 0.005, which is shown in figure 1. Figure 2 shows that most of the patients were from OPD 177(57.0%) and 131 (43.0%) were from IPD. Cases of UTI were higher in OPD as compare to IPD.

Table 1: Total number of microorganisms isolated from urine sample

Microorganisms	No. of isolates	
<i>E. coli</i>	288	45.6
<i>Klebsiella</i> species	20	31.5
Other gram - bacteria	158	25
Gram + bacteria	114	18
Fungus	51	8
Total	631	24.5

Patient of all age groups were enrolled in the current research. The causative agents (*E. coli* and *K. species*) of UTI were most commonly found in the age group between 19-35 years as compare to different other age group of patients (Table2). Table 3 shows that both ESBL/non ESBL isolates were recovered with wide range from the age group between 19-35 years and least with 0-1 years of age. Figure 3 shows that total cases of UTI were 631. Out of 631 UTI cases

308 cases were caused by *E. coli* and *K. species*. Among which ESBL producing bacteria were 213(69.0%) and non ESBL producing bacteria were 95 (31.0%). ESBL isolates were more as compare to non ESBL isolates recovered from cases of UTI.

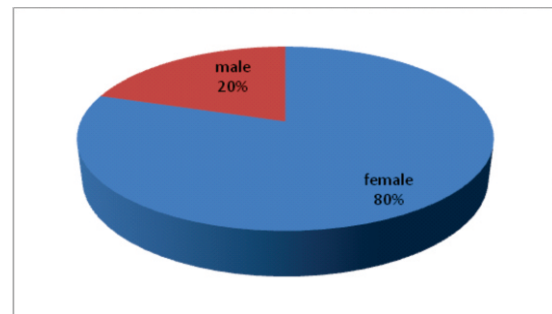


Figure 1: sex wise case distribution (p*0.005)

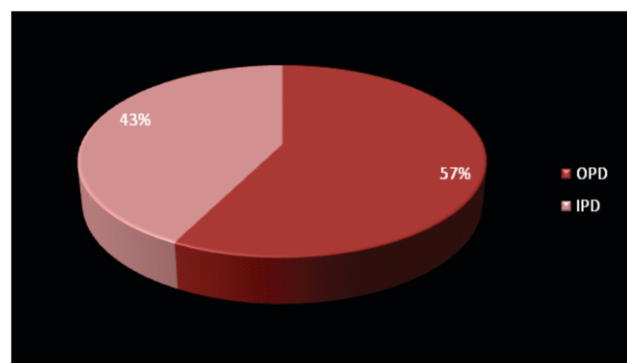


Figure 2: Case distribution according to the IPD and OPD (P* 0.036)

Table 4 shows that out of 213 ESBL isolates, 203 (70.5%) isolates of *E. coli* and 10 isolates of *Klebsiella* species (50.0%) were confirmed as ESBL producer. According to sex the ESBL producing isolates were higher in female 161 (65.4%) as compare to male 52 (83.9%) and non ESBL producing isolates were also higher in female 85 (34.6%) as compare to male 10 (16.1%) (Table5). Table 6 shows that numbers of ESBL/non ESBL producing isolates were recovered more from the OPD 114 (64.4%)/63 (35.6%) as compare to IPD 99 (75.6%)/32 (24.4%). Figure 4 showed that all most all the *E. coli*/*K. species* were sensitive to nitrofurantoin (Nit) 277 (94.5%)/16 (5.5%), gentamycin (Gen) 238 (92.6%)/19 (7.4%) and norfloxacin (Nx) 121(89.6)/14 (10.4%). No ESBL producing *E. coli* and *K. species* were found to resistant to meropenem. Similarly, among other antibiotic tested lower rate of resistance was seen towards the piperacillin/tazobactum (Ptz), ampicillin/salbactum (A/S) and amikacin (Ak) (Figure 5).



Table 2: Age wise case distribution

Age Groups	ORGANISMS		P value
	<i>E. coli</i>	<i>K. species</i>	
-1 yrs	10	0	0.215
0	100.0%	0.0%	
-18 yrs	42	0	
2	100.0%	0.0%	
19-35yrs	124	11	
	91.9%	8.1%	
36-60 yrs	66	7	
	90.4%	9.6%	
above 60 yrs	46	2	
	95.8%	4.2%	
Total	288	20	
	93.5%	6.5%	

Table 3: Age wise distribution of ESBL/ non ESBL isolates

Age Groups	ESBL/ non ESBL		P value
	Non ESBL	ESBL	
0-1 yrs	1	9	0.033
	10.0%	90.0%	
2-18 yrs	9	33	
	21.4%	78.6%	
19-35yrs	51	84	
	37.8%	62.2%	
36-60 yrs	25	48	
	34.2%	65.8%	
above 60 yrs	9	39	
	18.8%	81.2%	
Total	95	213	
	30.8%	69.2%	

Discussion

In hospital setting and community based UTI are second most common bacterial infectious diseases in human being. Misuse and overuse of antimicrobials in community and in hospital set up will not only be expensive to the patients but also increase the drug resistance in future. Mostly in hospital, ICU patients are more susceptible to infection and chances of bacterial colonization are high by various pathogens due to weak immune system [8]. ESBL producing organisms have emerged as major global problem and affect the treatment with broad spectrum antibiotics which may cause drug resistance as a serious problem in future [9]. The ESBL producers from urinary isolates in our study were (69.2%) but it was half less, (31.6%) in a study conducted by Chatterjee M et al [10].

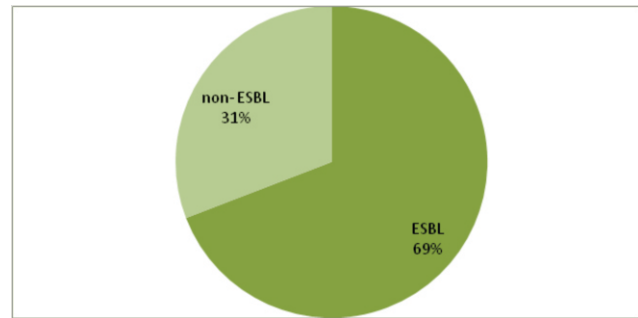


Figure 3: Total no. of ESBL and non ESBL isolated from cases of UTI Distribution of ESBL and non ESBL producing *Escherichia coli* and *Klebsiella* species.

Table 4: Distribution of ESBL and non ESBL producing *Escherichia coli* and *Klebsiella* species (p-0.055)

Microorganisms	ESBL	percentage	Non ESBL	Percentage
<i>Escherichia coli</i>	203	70.5	85	29.5
<i>Klebsiella</i> species	10	50.0	10	50.0
Total	213	69.2	95	30.8

Table 5: Sex wise distribution of ESBL and non ESBL isolates

Sex	ESBL	Non ESBL	Total
Female	161(65.4%)	85(34.6%)	246(79.9%)
Male	52(83.9%)	10(16.1%)	62(20.1%)

This is due to not proper using the antibiotics including 3rd generation cephalosporins and not follow the standard infection control practice in hospital as well as in community. In the present study, total numbers of positive urine samples were 631 processed from the OPDs and IPDs. Among which, 308 urine sample showed growth of *Escherichia coli* species 288(93.5%) and *Klebsiella* species 20(42.5%) were recovered from cases of UTI which was very similar to study conducted by Nepal K et al [11]. In our study, the cases of UTI were higher in female 246(79.9%) as compared to male 62(20.1%). Male: female ratio was 1:3.9. Female is more prone for UTI as compared to male due to the anatomical structure of female urogenital system [8]. Females showed a higher rate of ESBL producing among the *E. coli* (70.5%) and *K. species* (50.0%), which was similar to that of Ahmed SM et al [12]. This study revealed a higher occurrence of ESBL producing uropathogens in the adult age group of 19–35 years, which was similar to study done by Chander A et al [13].



Table 6: Distribution of ESBL and non ESBL according to IPD and OPD p- 0.036

Ward	ESBL	Non ESBL	Total
OPD	114(64.4%)	63(35.6%)	177(57.5%)
IPD	99(75.6%)	32(24.4%)	131(42.5%)

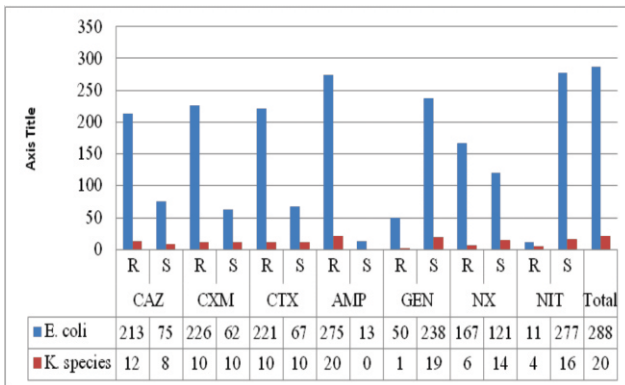


Figure 4: Antibiotic susceptibility of E. coli and K. species isolates

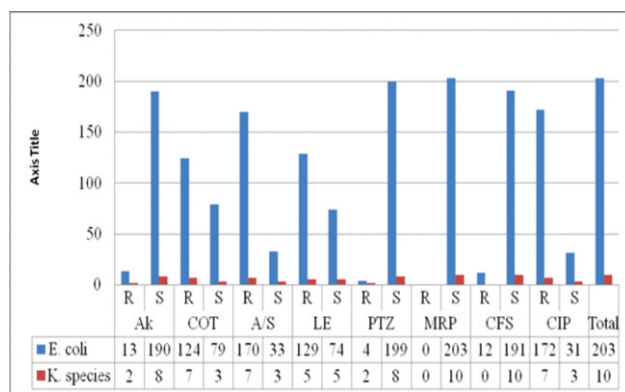


Figure 5: Antibiotic profile of ESBL producing E. coli and K. species isolates

The studies by Spanu T et al [14] showed ESBL production was higher in *K. pneumoniae* strains, as compared to *E. coli* isolates. Which was contrast to our study has shown increased number of ESBL producing *E. coli* was (70.5%) as compared to *K. species* (50.0%). Higher numbers of isolates were from outpatient department (57.5%) as compared to inpatient department (42.5%) and which was similar to the study conducted by Nepal K et al [11]. In present study numbers of ESBL/non ESBL producing isolates were recovered more from the OPD 114(64.4%)/63 (35.6%) as compared to IPD 99(75.6%)/32(24.4%). This is uncommon, very alarming situation and shows the trend of dissemination of ESBL producing bacteria to the community. This correlates well with study

conducted by Bonnet R et al showed that higher number of ESBL-producing isolates from outpatients was *E. coli* (57.8%) [15]. *Escherichia coli* accounts for a large number of urinary isolates as well as higher numbers of ESBL production than *K. pneumoniae* [16]. This is similar to our study. The increase in ESBL production among the clinical isolates is found to be ranging from <1 to 74.0% worldwide [11]. The probable reason for gradual increase in ESBLs production in various parts of world due to the random and inappropriate use of 3rd generation cephalosporins, which contribute to the evolution of ESBLs, not using of standard infection control practices in healthcare facilities and lack of national antibiotic policy. We found in our study ESBLs producing isolates were 100.0% susceptible to meropenem which confirms clearly with the CDC (1999) ESBLs definition and study conducted by Raut S et al [17]. All ESBL producing *E. coli* and *K. species* were found (100.0%) susceptibility to meropenem. Similarly, other antibiotic also showed increased susceptibility towards the piperacillin/tazobactam (97.2%), cefoperazone/sulbactam (94.4%) and amikacin (93.0%), and which was similar to study conducted by Singh N et al [18] and Iroha IR et al [19]. The ESBL producing organisms are increasing rapidly and becoming a major problem in the area of infectious diseases which includes multi drug resistance, difficulty in detection and treatment and increase in mortality of patients. As compared to other available antimicrobial agents, carbapenemes are the drug of choice and are reliable for treatment for infections caused by ESBL isolates. However, overuse and misuse of carbapenemes may also lead to drugs resistance among the other gram-negative bacteria. Therefore, knowledge of drug susceptibility, rational of drugs use and proper use of antibiotics including third-generation cephalosporins, must take effective measures to control infection in hospital environment as well as in a community. These methods are the most effective means of controlling and preventing the spread of ESBL producing microorganisms.

Conclusion

ESBL are clinically significant and when detected, indicate the need for the use of appropriate antibacterial agents. Infection with strains expressing ESBLs is a great challenge for both microbiologists and clinicians as they are having less therapeutic options. In this study infection with ESBL production among the *E. coli* and *K. species* isolated from urine sample are high and creating serious problem in country like Nepal. Study also observed that high numbers of ESBL



isolates were recovered from OPD which is uncommon and serious issue indicating that there is dissemination of ESBL producing *E. coli* and *K. species* in the community. Meropenem is the only drug of choice against the ESBL isolates. So it should be made mandatory and very important to have routine monitoring system to detect the ESBL producing isolates in clinical laboratories.

References

- [1] Harakuni S, Mutnal Sastry AS and Bhat KS, Essentials of Medical microbiology. Antimicrobial agents, antimicrobial resistance and antimicrobial susceptibility testing, 1st ed. 2016:83.
- [2] Behzadi P, Behzadi E, Yazdanbod H, Aghapour R, Akbari Cheshmeh M, Salehian Omran D. A survey on urinary tract infections associated with the three most common uropathogenic bacteria. *Maedica (Buchar)*. 2010;5(2):111–5. PMID: PMC3150015 PMID: 21977133
- [3] Cox CE. Nosocomial urinary tract infections, *Urol*, 32:3 (1988) 210-15. DOI:10.1016/0090-4295(88)90386-x
- [4] Gniadkowski M, Evolution and epidemiology of extended-spectrum beta-lactamases (ESBLs) and ESBL-producing microorganisms, *Clin Microbiol Infect*, 7:11 (2001) 597-608. DOI: <https://doi.org/10.1046/j.1198-743x.2001.00330.x>
- [5] Cheesbrough M, District laboratory practice in tropical countries, part II. 2nd ed. New York: Cambridge University Press; 2006.
- [6] Clinical and Laboratory Standards Institute. CLSI document M100-S25, Performance standards for antimicrobial susceptibility testing: Twenty fifth informational supplement ed. Wayne: CLSI; 2015 Vol. 34 No. 1. ISBN- 15623874219781562387426.
- [7] Jenkins SG, Schuetz AN. Current concepts in laboratory testing to guide antimicrobial therapy. *Mayo Clin Proc*, 87:3 (2012) 290-308. doi: 10.1016/j.mayocp.2012.01.007.
- [8] Karadesai S and Metgud S, Prevalence of extended spectrum β - lactamase- producing clinical isolates of *Klebsiella pneumoniae* in intensive care unit patients of a tertiary care hospital, *Ann Trop Med public Health*, 4 (2011) 96-8. DOI: 10.4103/1755-6783.85760
- [9] Sharma M, Prevalence and antibiogram of Extended Spectrum β -Lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing *Escherichia coli* and *Klebsiella* species, *J Clin Diagn Res*. 10 (2013) 2173-77. Doi: 10.7860/JCDR/2013/6460.3462.
- [10] Chatterjee M, Banerjee M, Guha S, Lahiri A, Karak K, Prevalence of ESBL producing urinary isolates and their drug resistance pattern in eastern part of India, *Sri Lankan J Infect Dis*, 2:1 (2012) 36-41. DOI: <http://dx.doi.org/10.4038/sljid.v2i1.3716>
- [11] Nepal K, Pant ND, Neupane B, Belbase A, Baidhya R, Ram Krishna Shrestha RK, Extended spectrum beta-lactamase and metallo beta-lactamase production among *Escherichia coli* and *Klebsiella pneumoniae* isolated from different clinical samples in a tertiary care hospital in Kathmandu, Nepal, *Ann Clin Microbiol Antimicrob*, 16:62 (2017)1-7. <https://doi.org/10.1186/s12941-017-0236-7>
- [12] Abujnah AA, Zorgani A, Sabri MA, El-Mohammady H, Khalek RA, Ghenghesh KS, Multidrug resistance and extended-spectrum β -lactamases genes among *Escherichia coli* from patients with urinary tract infections in Northwestern Libya, *Libyan J Med*, 10(2015)26412. PMID: 25651907
- [13] Chander A and 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 Res Notes*, 6 (2013) 487. doi:10.1186/1756-0500-6-487
- [14] Spanu T, Luzzaro F, Perilli M, Amicosante G, Toniolo A, Fadda G, The Italian ESBL Study Group. Occurrence of extended spectrum β -lactamases in members of the family Enterobacteriaceae in Italy: implications for resistance to β -lactams and other antimicrobial drugs, *Antimicrob Agents Chemother*, 4 (2002) 196-202. DOI: 10.1128/AAC.46.1.196-202.2002
- [15] Bonnet R, Growing group of extended-spectrum β -lactamases (ESBLs) the CTX-M enzymes, *Antimicrob Agents Chemother*, 48:1 (2004) 1-14. DOI: 10.128/AAC.48.1.1-14.2004
- [16] Moyo SJ, Aboud S, Kasubi M, Lyamuya EF, Maselle SY, Antimicrobial resistance among producers and non-producers of extended spectrum β -lactamases (ESBLs) in urinary isolates at a tertiary Hospital in Tanzania, *BMC Res Notes*, 3:1 (2010) 348. DOI: 10.1186/1756-0500-3-348
- [17] 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*, 5:2 (2015) 69-75. DOI: 10.5799/ahinjs.02.2015.02.0179
- [18] Singh N, Pattnaik D, Neogi DK, Jena J, Mallick B, Prevalence of ESBL in *Escherichia coli* Isolates among ICU Patients in a Tertiary Care Hospital, *J Clin Diagn Res*, 10:9 (2016) 19-22. doi: DOI: 10.7860/JCDR/2016/21260.8544
- [19] Iroha IR, Okoye E, Osigwe CA, Moses IB, Ejikeugwu CP, (2017) Isolation, Phenotypic Characterization and Prevalence of ESBL producing *Escherichia Coli* and *Klebsiella* species from Orthopedic Wounds in National Orthopedic Hospital Enugu (NOHE), South East Nigeria, *J Pharma Care Health Sys*, 4 (2017) 184. DOI: 10.4172/2376-0419.1000184

