Antibiotic Profile of Extended Spectrum Beta Lactamase Escherichia Coli from Clinical Samples

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ABSTRACT

Background: Extended spectrum β-lactamases have addressed the serious challenges worldwide due to the emergence of ESBL producing genes which possess a serious threat for the treatment of infections both in community and hospitals since it is found to be increasing trends of multidrug resistance. This study was focused to find out the antibiotic profile of multidrug resistant Escherichia .coli and status of ESBLs producing E.coli.

Methods: This was a cross-sectional study conducted over a period of 2 years (September 2017 to April 2019) at microbiology laboratory of Nepal Mediciti Hospital. A total of 16542 samples were processed. Various clinical samples were collected from both inpatients and outpatients aseptically and without contaminating skin commensals. Standard microbiological techniques were used for isolation and identification of pathogens. Extended spectrum beta-lactamases were phenotypically confirmed by combined disc method.

Results: Out of 1449 E.coli isolates, 323(22.29%) were found to be MDR E.coli. Isolation rate of ESBL producing E.coli (66.56%) were found to be high among MDR E.coli isolates.

Conclusion: There was increasing prevalence of ESBL producing E.coli and was essential to monitor antibiotic susceptibility pattern and formulate antibiotic policy to prevent the spread of MDR and ESBL producers.

Keywords: E.coli, Extended spectrum β-lactamase, Multidrug resistant

Access this article Online		Article Info.
Quick Response Code	Website:	How to cite this article in Vancouver Style?
View PDF	www.jkahs.org.np	Chaudhary MK, Jadhav I, Banjara MR. Antibiotic Profile of Extended Beta Lactamase Escherichia Coli from Clinical samples. Journal of Karnali Academy of Health Sciences 2020;3(2): 102-110
	DOI: https://doi.org/10.3126/jkahs.v3i2.30840	Received : 12 May 2020 Accepted : 8 August 2020 Published Online : 9 August 2020 Conflict of Interest : None Source of Support : None

INTRODUCTION:

Extended-spectrum beta-lactamases (ESBLs) are the group of beta-lactamase enzymes, which hydrolyze and cause resistance to the oxyimino-cephalosporins (cefotaxime,ceftazidime,ceftriaxone,cefuroxime and cefepime) and monobactams(aztreonam), but not the cephamycins (cefoxitin and cefotetan) or carbapenems (imipenem, meropenem, and ertapenem), produced by *Escherichea coli* and *Klebsiella pneumoniae*.[1]

Beta-Lactam antibiotics include (penicillins, cephalosporin, carbapenems & monobactams) are common to treat bacterial infections. The emergence of β-Lactamases mediated bacterial resistance, are due to the irrational use of antibiotics, particularly the third generation cephalosporins, which subsequently led to the development of ESBL producing bacteria.[2]

Emergence of resistant bacteria worldwide possess a great threat to treatment outcomes in community and hospital settings. *E. coli* is one of the commonest pathogen to exhibit multidrug resistance. Important risk factors for infection with MDR and ESBL *Escherichia coli* are prolonged antibiotic exposure, overstay in hospital, irrational use of 3rd generation cephalosporins, severe illness,frequent use of IV devices and catheters.[3]

Extended spectrum beta lactamases are plasmid mediated enzymes which confer bacterial resistance to the penicillins, first, second and third generation cephalosporins and aztreonam.[4]

The origin of Extended spectrum beta lactamases from mutant forms of broad spectrum beta lactamases such as the TEM-1,TEM-2 and SHV-1 enzymes coded by genes located on transferrable plasmids, which can easily spread form one organism to another.[5]The epidemiology of ESBL-producing bacteria has been increasing day by day with crossing boundaries between hospitals and the community. The acquisition of efficient mobile elements has accelerated the transfer of various antibiotic resistance genes. Continuous surveillance system to monitor multidrug-resistant bacteria is urgently required worldwide.[6]Current knowledge of prevalence of ESBL producing strains and antibiotic status of MDR, ESBL strains will help global awareness for the formulation of antibiotic policy and antibiotic stewardship program to optimize antibiotic use for improving patient outcomes, reducing adverse consequences and provide cost-effective therapy. Therefore, the present study was conducted with an objectives of studying the antibiotic profile of MDR and ESBL *Escherichia coli* producing strains which helps to formulate antibiotic policy and antibiotic stewardship program for the prevention and control of infections.

MATERIAL AND METHODS

This study was a cross sectional study conducted in Microbiology Laboratory of Nepal Mediciti Hospital, Bhaisepati; Nepal from September 2017 to April 2019.This study was approved by Institutional Review Committee of Nepal Mediciti Hospital. A total of 16542 samples were processed. Various clinical samples were collected from both inpatients and outpatients aseptically and without contaminating skin commensals. Urine samples were inoculated on CLED agar whereas pus, sputum, swab ,biopsy, fluid , foley'stip, vaginal swab, catheter tip, CVP tip, BAL, suction tube, bile were inoculated aseptically on blood agar, chocolate agar and Mac-Conkey agar and incubated aerobically at 37±°C for 24-48 hours. About 8-10 ml of blood from adult patients and 1 - 3 ml of blood from pediatric cases were inoculated in BACTEC ™ plus Aerobic/F vial and BACTEC™ PEDS PLUS/F vial respectively. The culture bottles were incubated at 37°c overnight (at least 5 days) inside BACTEC ™ FX and checked for the turbidity or any visual change daily. The specimens were sub cultured on Blood agar, chocolate agar and MacConkey agar plates and incubated aerobically at 37±°C for 24 hours using a standard operative procedures. Significant bacterial count (10^5CFU/ml) was recorded from urine culture isolates. The identification of bacterial isolates were carried out by cultural, morphological characters, Gram stain and appropriate biochemical tests (triple sugar iron, indole, citrate, urease and motility) following standard procedures.

Antibiotic Susceptibility Tests

Modified Kirby-Bauer disc diffusion method was applied to perform Antibiotic susceptibility test (AST) recommended by Clinical and Laboratory Standard Institute (CLSI).[2] The inoculums were prepared in nutrient agar by taking 3-5 identical colonies of *Escherichia coli* that matched to 0.5McFarland standard turbidity. A sterile cotton swab was dipped into inoculum after 15 minutes and streaked over dried surface Muller Hinton agar (MHA) plate. The following commercially available antibiotic discs used for urine culture were amikacin (30µg), gentamycin (10µg), ciprofloxacin (30µg), ceftriaxone (30µg), cefotaxime (30µg), ceftazidime (30µg), nitrofurantoin (300µg), norfloxacin (10µg), nalidixic acid (30µg) and ofloxacin (5µg).For other clinical samples, antibiotic discs applied were amikacin (30µg), gentamycin (10µg), ciprofloxacin (30µg), ceftriaxone (30µg), cefotaxime (30µg), ceftazidime (30µg), cotrimoxazole (25µg), cefixime(5µg), cefepime (30µg), tigecycline (15µg), imipenem (10µg), meropenem (10µg) polymyxinb (300µg) and colistin (10µg). Plates were incubated aerobically at 37°C for 24 hours. Zone diameter in millimeters was measured and organisms were identified as sensitive, resistant and intermediate as per CLSI 2013 guidelines. *Escherichia coli* strain ATCC25922 was used as control strain.

Screening of ESBL

3rd generation cephalosporins (ceftazidime, cefotaxime and ceftriaxone) were used to screen ESBL producers. Isolates resistant to more than one of these agents were identified as possible ESBL producers.[7, 8]Confirmation of ESBL by confirmatory test following CLSI guidelines.

Confirmatory Test for ESBL

ESBL detection was confirmed by two phenotypic methods.

Double disc synergy test (DDST)

Microbial inoculums matched with 0.5 McFarland Standard turbidity were inoculated on dried MHA plates. Antibiotic disc of amoxicillin-clavulanate(20µg/10µg was placed at the center and ceftazidime(30µg) and cefotaxime(30µg) were placed on either side of 30mm apart from center to center. Plates were incubated at 37°C for 18-24 hours. Those inoculums which exhibited an enhanced zone of inhibition in between amoxicillin/clavulanic acid and ceftazidime and cefotaxime were identified as confirmed ESBL producers.[9, 10]

Combined disc method

Ceftazidime (30µg) alone and ceftazidime with clavulanic acid(30µg/10µg)and cefotaxime (30µg) and cefotaxime with clavulanic acid (30µg/10µg) were placed at an appropriate distance from each other on inoculated MHA plate. The plate was incubated at 37°C for 18-24 hours aerobically. Inhibition zone differences by ≥5mm in either ceftazidime clavulanic acid with ceftazidime alone or cefotaxime clavulanic acid with cefotaxime alone was interpreted as confirmed ESBL.[11]The quality control was done using *Escherichia coli* ATCC 25922 as negative control.

Statistical Analysis

The data was entered and percentage calculation were analyzed by using Statistical Package for Social Sciences (SPSS) version 21 software.

RESULTS

Total of 16542 samples were collected in which 1449 *Escherichia coli* isolates were recovered from various clinical samples of inpatients and outpatients of the hospital. The highest number of Escherichia coli was isolated from urine followed by sputum, swab,pus,blood,fluid,foley's tip,vaginal swab, catheter tip,BAL,biopsy,bile suction tube,CVP tip,ET tube. Distribution of *Escherichia coli* isolates on the basis of source. (Fig.1)

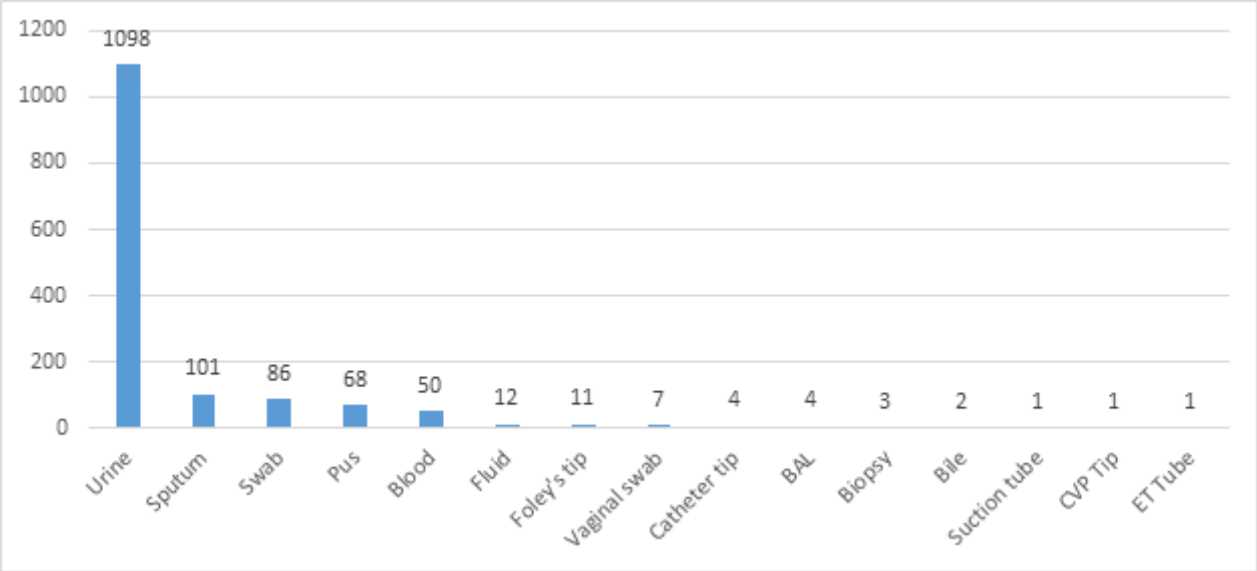

Fig.1.Source wise distribution of *Escherichia coli* isolates

Antimicrobial susceptibility test of *Escherichia coli* isolates from various clinical samples i.e. urine, sputum, swab, pus and blood were shown Table1-2. In the present study, it is observed that

Escherichia coli isolates from urine culture is 96.54% to nitrofurantoin followed by amikacin (80.78%). Most of *E.coli* from urine were resistant to third generation cephalosporins indicates possible ESBL producers.

Similarly, *Escherichia coli* isolates from sputum, swab, pus and blood were found to be 100% sensitive to tigecycline followed by colistin and polymyxin b. Resistance to third

generation cephalosporins is between 26% and 93%, which is highly variable. Whereas, resistant pattern of carbapenem is between 4% and 20% which is quite low. Isolates resistant to third generation cephalosporins were considered as possible ESBL producers

Table 1: Antibiotic susceptibility pattern of *E.coli* from urine

Antibiotics	Antibiotic susceptibility rate No (%) *E.coli*(1098)	
	Sensitive	Resistant
Amikacin(AK)	887(80.7)	211(19.3)
Gentamycin(G)	812(73.9)	286(26.1)
Ciprofloxacin(CIP)	511(46.5)	587(53.5)
Ceftriaxone(CTR)	646(58.8)	452(41.2)
Cefotaxime(CTX)	646(58.8)	452(41.2)
Ceftazidime(CAZ)	646(58.8)	452(41.2)
Nitrofurantoin(NIT)	1060(96.5)	38(3.5)
Norfloxacin(NX)	498(45.4)	600(54.6)
Nalidixic acid(NA)	258(23.5)	840(76.5)
Ofloxacin(OF)	493(44.8)	605(55.2)
Co-trimoxazole(COT)	586(53.4)	512(46.6)

Table 2: Antibiotic susceptibility pattern of *E.coli*

Antibiotics	Sputum		Swab		Pus		Blood	
	n=101 No (%)		n=86 No (%)		n=68 No (%)		n=50 No (%)	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
AK	44(43.5)	57(56.5)	57(66.3)	29(33.7)	59(86.7)	9(13.3)	46(92)	4(8)
G	42(41.5)	59(58.5)	61(70.9)	25(29.1)	57(83.8)	11(16.2)	41(82)	9(18)
CIP	15(14.8)	86(85.2)	49(56.9)	37(43.1)	42(61.7)	26(38.3)	37(74)	13(26)
CTR	24(23.7)	77(76.3)	33(38.4)	53(61.6)	31(45.6)	37(54.4)	39(78)	11(22)

CTX	15(14.8)	86(85.2)	49(56.9)	37(73.1)	41(60.3)	27(39.7)	37(74)	13(26)
CAZ	24(23.7)	77(76.3)	61(70.9)	25(29.1)	31(45.6)	37(54.4)	39(78)	11(22)
COT	23(22.7)	78(77.3)	43(50)	43(50)	35(51.4)	33(48.6)	34(68)	16(32)
CFX	7(6.9)	94(93.1)	61(70.9)	25(29.1)	48(70.5)	20(29.5)	32(64)	18(36)
CPM	11(10.8)	90(89.2)	61(70.9)	25(29.1)	48(70.5)	20(29.5)	39(78)	11(22)
TGC	101(100)	-	86(100)	-	68(100)	-	50(100)	-
IPM	86(85.2)	15(14.8)	68(79.1)	18(20.9)	59(86.7)	9(13.3)	45(90)	5(10)
MRP	94(93.1)	7(6.9)	81(94.2)	5(5.8)	62(91.2)	6(8.8)	48(96)	2(4)
PB	100(99.2)	1(0.8)	85(98.8)	1(1.2)	66(97.1)	2(2.9)	50(100)	-
CL	101(100)	-	85(98.8)	1(1.2)	67(98.5)	1(1.5)	50(100)	-
C*	-	-	-	-	-	-	45(90)	5(10)

Note: AK-Amikacin, G-Gentamycin, CIP-Ciprofloxacin, CTX-Ceftazidime,CAZ-Ceftazidime,COT-Cotrimoxazole CFX-Cefixime, CPM-Cefepime, TGC-Tigecycline, IPM-Imipenem, MRP-Meropenem, PB-Polymyxin b, CL-Colistin

*Note: * C-Chloramphenicol used in blood culture*

Of the 1449 total *E.coli* isolates, 323/1449(22.29%) isolates were multi -drug resistance.Out of the total MDR *Escherichia coli* isolates, 215/323(66.56%) isolates were ESBL producers by combined disc method. However, maximum number of ESBL production was shown by using ceftazidime/ ceftazidime: clavulanic acid (CAZ/CAZC) combination. The maximum number of ESBL *Escherichia coli* was isolated from urine 194(90.23%), followed by sputum 12(5.58%), swab5 (2.32%), pus 2 (0.93%) and blood 2 (0.93%) as shown in table 3.

Antibiotic susceptibility pattern of ESBL *E.coli* producers showed similar pattern of sensitivity to tigecycline (100%) followed by polymyxin b, colistin and meropenem as non-ESBL producing *E.coli*. But the resistant to third generation cephalosporins showed increasing trends as compared to non-ESBL producers (table 4).

Table 3: Distribution of MDR and ESBL *E.coli* from clinical samples

Specimen	MDR E. coli No. (%)	ESBL *E.coli* No (%)
Urine	255(78.95)	194(90.23%)
Sputum	46(14.24)	12(5.58%)
Swab	13(4.1)	5(2.32%)
Pus	5(1.54)	2(0.93%)
Blood	4(1.24)	2(0.93%)
Total	323(100.0)	215(100.0)

Table 4: Antibiotic susceptibility pattern of ESBL *E.coli*

Antibiotics	Antibiotic susceptibility rate No (%) ESBL *E.coli*(215)	
	Sensitive	Resistant
Amikacin(AK)	197(91.6)	18(8.4)
Gentamycin(G)	180(83.7)	35(16.3)
Ciprofloxacin(CIP)	125(58.2)	90(41.8)
Ceftriaxone(CTR)	-	215(100)
Cefotaxime(CTX)	1	214(97.3)
Ceftazidime(CAZ)	-	215(100)
Nitrofurantion(NIT)*	182(93.8)	12(6.2)
Norfloxacin(NX)*	109(56.2)	85(43.8)
Nalidixic acid(NA)*	9(4.6)	185(95.4)
Ofloxacin(OF)*	91(46.9)	103(53.1)
Tigecycline(TGC)	215(100)	-
Imipenem(IPM)	148(68.8)	67(31.2)
Meropenem(MRP)	194(90.2)	21(9.8)
Polymyxin B(PB)	215(100)	-
Colistin(CL)	215(100)	-

Note: * used in urine culture

DISCUSSION

Despite the discovery of antibiotics, emergence of MDR and ESBLs producing bacteria due to the extensive use of extended spectrum cephalosporins (ESCs) since early 1980's is a significant evolution

in antimicrobial resistance. Several other factors including misuse of drugs, inappropriate antibiotic treatment, extensive use of antimicrobials has also contributed to the emergence of drug resistant bacteria. The present study was conducted in the department of microbiology laboratory, Nepal Mediciti Hospital during a period of September 2017 to April 2019 with the aim of understanding the antibiotic profile of MDR and ESBL producing *Escherichia coli.*

The present study documented that the highest number of *E.coli* isolates were recovered from urine (n=1098(75.77).With regard to urinary tract infection, *E.coli* showed great extent of resistance to nalidixic acid, co-trimoxazole and third generation cephalosporins. Similar pattern of resistant in urinary isolates of *E.coli* was shown in Nepal and India.[12,13,14.] In contrast to our result, Perez et.al reported *E.coli* isolates were 94% resistant to ceftriaxone.[15]This may be due to the irrational use of third generation cephalosporins.[16] However; a significant degree of susceptibility was found to nitrofurantoin (96.5%) followed by amikacin (80.7%) and gentamycin (73.9%).Similar findings have been reported in various studies.[12,13,14,17,11,18].This may be due to the rational use of these drugs in UTIs cases since it is reserved drug for UTIs.

In this study, analysis of antibiotic susceptibility of *E.coli* isolated from sputum, blood, swab, pus demonstrated a significant degree of susceptibility towards tigecycline (100%) followed by colistin (98% to 100%), polymyxinb (97% to 100%), meropenem (91% to 96%) and imipenem (79% to 90%).Similar results were shown in other studies.[14,19.] It was found to be higher resistant pattern of cephalosporins (22% to 93%), fluoroquinolones (26% to 85%),aminoglycosides (8% to 59%) as compared to urine isolates. Several studies conducted in Nepal showed similar results.[12,19,20] In contrast to our study, Bamford et al.noted higher susceptibility pattern towards cephalosporins, fluoroquinolones, aminoglycosides.[21] The increased level of drug resistance is a major concern worldwide since these are the first line drugs recommended internationally[22,23] and are irrational used in public and private sectors.[24,25.]

The present study noted (323/1449)22.29% MDR *E.coli* isolates that were suspected of being ESBL producers were confirmed by combined disc method. Prevalence of ESBL *E.coli* was (215/323)66.56% which was alarming high. Several studies reported high prevalence i.e.40-70% of ESBL *E.coli* among MDR *E.coli*.[11,19,26-29] Kashyap et.al reported 37% ESBL*E.coli*.[30] But the study conducted by Anil chander et.al in 2013 observed only 13.51% ESBL prevalence in *E.coli* which is analogous result to other study.[31,32.]This is not similar with our study due to the variation in geography, study design and selection of type of antimicrobial agents. The indiscriminate use of beta-lactam antibiotics leads to the generation of selective pressures which have led to the selection of a variety of mutated forms of beta lactamases.[33.]Antibiotic profile of ESBL producing *E.coli* were found to be higher sensitivity towards tigecycline (100%), polymyxin B (100%), colistin (100%) followed by amikacin (91.6%), meropenem (90.2%) and imipenem (68.8%).Susceptibility to nitrofurantoin was 93.8% against ESBL producing *E.coli* isolated form urine. So, it is the drug of choice for treating infection caused by ESBL producing *E.coli.* The result of similar study conducted in Nepal and India.[34, 14, 8.]High resistant rates were observed to cephalosporins, nalidixic acid followed by fluoroquinolones. Similar findings were reported by Al-Zarouni et al.[35]

CONCLUSION

High prevalence of extended spectrum beta lactamase (ESBL) *E.coli* was observed. No resistance was documented to tigecycline, polymyxin b, and colistin suggesting the suitable drug of choice for treating ESBL producing *E.coli* causing life threatening infections. Of particular concern, proper formulation of antibiotic policy and antibiotic stewardship program absolutely required in each and every health sectors by all concern authorities. This study forwarded a real massage to all the clinicians for the emergence of XDR and PDR resistant bacteria in near future, if past experience with MDR and ESBLs was any indicator.

Acknowledgement: We would like to acknowledge Nepal Mediciti Hospital, Nepal for providing ethical approval and research work. We also thank to the microbiology laboratory staffs for their valuable support to complete this work.

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