Biofilm Production among Extended Spectrum Beta Lactamase Producing and non-Producing *Escherichia coli* Isolated from Clinical Specimens

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ABSTRACT

Objectives: To determine prevalence of ESBL producing and non-producing *Escherichia coli* in different clinical specimens and biofilm production along with antimicrobial susceptibility pattern of the bacteria.

Methods: The study was carried out in Sukraraj Tropical Infectious Disease Hospital, Teku, Kathmandu from September 2019 to February 2020. *E. coli* was identified by macroscopic, microscopic and biochemical characteristics. The AST was performed by Kirby Bauer's disc diffusion method and ESBL production was detected by using Ceftriaxone disc metod. Biofilm detection of the isolates was done by TCP technique.

Results: Out of 179 gram-negative isolates, 76(42.46%) were found to be *E. coli*. Out of 76 isolates, 22(28.95%) were ESBL producing and 54(71.05%) were ESBL non-producing. Among 22 ESBL producer, 8(36.36%) was found to be biofilm producer whereas among 54 ESBL non-producer 13(24.07%) was found to be biofilm producer. Biofilm producing ESBL producers were found to be sensitive towards Colistin (100%), Carbapenems (87.5%) and Nitrofurantoin (87.5%) whereas biofilm producing ESBL non-producer was found to be sensitive towards Colistin (100%) Carbapenems (100%) and Nitrofurantoin (100%).

Conclusion: For the treatment of ESBL infection, currently carbapenems are the drug of choice. Among ESBL producers and non-ESBL producers, biofilm production was high with 36.36% in ESBL producing isolates.

Keywords: *Escherichia coli*, antimicrobial susceptibility pattern, ESBL, biofilm, Nepal

INTRODUCTION

The β-lactamases are the major defense of bacteria against β-lactam antibiotics that inactivate these antibiotics by hydrolysis and result in effective compounds (Tooke et al 2019). Extended spectrum beta lactamase (ESBLs) are group of beta lactamases which share the ability to hydrolyze third generation cephalosporins and aztreonam yet are inhibited by clavulanic acid. The ESBLs are frequently plasmid encoded which carry genes encoding resistance to different drugs. Therefore, antibiotic options in treatment of ESBL producing organisms are extremely limited and hence the presence of ESBLs carries tremendous clinical significance (Paterson and Bonomo 2005).

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In addition to increasing rate of repeated antimicrobial resistance among clinical strains, antimicrobial resistance is an innate feature of bacterial biofilms that may further complicate patient treatment (Sanchez et al 2013). Within a biofilm, bacteria communicate with each other by production of chemotactic particles or pheromones, a phenomenon called quorum sensing that helps bacteria to share resistance character (Zubair et al 2011). High antimicrobial concentrations are required to inactivate organisms growing in a biofilm, as antibiotic resistance can increase 1000 folds (Stewart and Costerton 2001).

Biofilms are associated with many medical conditions including indwelling medical devices, dental plaque, upper respiratory tract infections, peritonitis, and urogenital infections (Reid G 1999). Biofilm serves as a protective shield to the microorganisms not only from altered pH, osmolarity, nutrients scarcity and mechanical forces but also block the access of bacterial biofilm communities from antibiotics and host immune cells which lead to the emergence of bad bugs infections like multi drug resistant, extensively drug resistant and totally drug resistant bacteria (Sharma et al 2019).

According to National Institute of Health, in US, biofilms account for up to 80% of the total number of microbial infections including endocarditis, cystic fibrosis, periodontitis, rhinosinusitis, osteomyelitis, non-healing chronic wounds, meningitis, kidney infections, and prosthesis and implantable device- related infections (Joo et al 2012). Biofilms facilitates horizontal gene transfer since the cells are maintained in close proximity to each other and are not fully immobilized and hence can exchange genetic material (Madsen et al 2012). Bacterial biofilm might get attached to the implanted medical devices or to the living surfaces during the course of infection (Donlan 2002).

The beta lactamases production and biofilm formation synergistically contribute for extensive dissemination of multi drug resistant strains of gram-negative bacilli. In addition, they are also responsible for posing a serious health crisis implicating chronicity, persistence and relapse of infections leading to high rate of morbidity and mortality (Dumaru et al 2019).

Though the studies on the multidrug resistance among the β- lactamase producing *E. coli* are abundant in Nepal but studies on biofilm which are strongly associated with β-lactamase producing *E. coli* are limited. This creates a strong necessity for such studies to be carried out to detect the biofilm producer among the *E. coli*. The outcome of the study will provide information of antibiogram profile of biofilm producing *E. coli* which can guide towards effective management of biofilm associated β-lactamase producing & non-producing *E. coli*.

MATERIALS AND METHODS

Study site, duration and study population

This study was carried out at Sukraraj Tropical Infectious Disease Hospital, Kathmandu where collection of data was done and processing of specimens was done in Med-Micro Research Laboratory. The laboratory work was conducted from September 2019 to February 2020. A total of 1509 samples were processed which includes 289 blood sample, 946 urine sample, 250 sputum sample, 5 pus, 10 throat swab, 5 CSF and 4 fluids sample.

Laboratory analysis

Culture of the specimen

The received specimens in laboratory were immediately culture on MA, BA, CA and CLED agar based on the nature of samples. The inoculums on plate was streaked with sterile inoculating loop to obtain discrete colonies. Then the plates were incubated at 37^{o}C for 24 hours. (Cheesbrough 2006).

Isolation and identification of isolates

For the identification of *E. coli*, standard microbiological procedures were followed as described in Bergey's Manual of Systematic Bacteriology. This involves morphological appearance of the isolated colony, staining reactions and biochemical properties. Each of the organism were first isolated in the pure form. Then isolates were identified using macroscopic, microscopic and performed biochemical test. Routine conventional laboratory techniques include gram staining, catalase, oxidase, O/F, Indole test, Methyl red test, VP test, Citrate utilization test, Triple Sugar Iron (TSI) test, Urease test, Motility test, Sulphur production test and Gas production test.

Antibiotic susceptibility test

The antibiotic susceptibility testing of *E. coli* to different antimicrobial disk was done by modified Kirby- Bauer disc diffusion method as recommended by Clinical Laboratory

Standard Institute (CLSI, 2017). The commercial antibiotics discs and concentration used were Amoxicillin (300mcg), Cefixime (15mcg), Ceftazidime (30mcg), Ceftazidime+ clavulanate (30+10 mcg), Ceftriaxone (30mcg), Ciprofloxacin (5mcg), Cotrimoxazole (25mcg), Gentamicin (10mcg), Imipenem (10mcg), Meropenem (10mcg), Nitrofurantoin (300mcg), Polymyxin B/Colistin (50mcg).

Laboratory detection of ESBL producing strains

Screening test for ESBL detection

Screening for ESBL production was done by testing the bacterial isolates against ceftriaxone disc (30µg) by using the standard disc diffusion method as recommended by the CLSI (2017). As directed by CLSI guidelines, the isolates that give ≤ 25mm diameter of zone of inhibition respectively were suspected to the ESBL producer and processed for confirmatory test of ESBL production.

Confirmatory test for ESBL detection

The Ceftazidime (30µg) disc alone and in combination with Clavulanic acid (ceftazidime + clavulanic acid 30/10 µg discs) were applied on a plate of MHA which was inoculated with the test strain and incubated for 16-24 hours. An increase in zone of diameter ≥ 5mm was considered to indicate as ESBL producer.

Screening of Biofilm Production

The ability of biofilm formation was tested by Tissue Culture Plate Technique. At first isolates preserved in Eppendorf tube was kept in room temperature and sub cultured in NA and incubated at 37°C for 24 hrs. Organisms isolated from fresh agar plates were inoculated in 10 ml of Trypticase soy broth (TSB) supplemented with 1% glucose and incubated at 37°C for 24 hr. The cultures were then diluted 1:100 with fresh medium. Individual wells of sterile 96 well flat bottom polystyrene tissue culture treated plates were filled with 200 µl of the diluted cultures. The control organisms were also incubated, diluted and added to tissue culture plate. Negative control wells contained TSB with 1% glucose. The plates were incubated at 37°C for 24 hr. After incubation, contents of each well were removed by gentle tapping. The wells were washed with 0.2 ml of phosphate buffer saline (pH 7.2) four times to remove free floating bacteria. Next is the step of fixation. Biofilm formed by bacteria adherent to the wells were fixed by 2% sodium acetate and then stained by crystal violet (0.1%). Excess stain was removed by using deionized water and plates were kept for drying. Optical density (OD) of stained adherent biofilm was obtained by using micro ELISA auto reader at wavelength 570 nm. The experiment was performed in triplicate and repeated three times (Hassan et al 2011).

The interpretation of biofilm production was done according to the criteria of Stepanovic et al (2007).

Average OD value	Biofilm production
≤ ODc / ODc < ~ ≤ 2x ODc	Non
2x ODc < ~ ≤ 4x ODc	Moderate
> 4x ODc	Strong

Optical density cut-off value (ODc) = average OD of negative control + 3x standard deviation (SD) of negative control.

Statistical Analysis

All the raw data were analyzed using MS Excel software version 2016. Chi square test was used to determine significant association of dependable variables to different independent variables.

RESULTS

In the study, a total of 1509 samples, which includes blood, sputum, urine, CSF, fluids (Pleural and ascitic), pus and throat swab were processed. Out of which 239 (15.84%) showed the culture positive and the rest 1270(84.16%) showed no growth.

Out of 239 culture positives sample, 179(74.90%) were gram-negative. Among 179 gram negative isolates, 76(42.46%) were found to be *E.coli*, 37(20.67%) were *Salmonella* species, 26(14.53%) were *Klebsiella pneumoniae*, 20(11.17%) *Pseudomonas*, 6(3.35%) were *Enterococcus*, 5(2.79%) were *K. oxytoca*, 3(1.67%) were *Acinetobacter* sps, 2(1.12%) were *Citrobacter diversus*, 2(1.12%) were *Morganella morganii* and 2(1.12%) was found to be *Neisseria* species.

Out of 76 *E. coli* isolates, 22(28.95%) were found to be ESBL producing and 54(71.05%) were ESBL non-producing.

Out of 76 *E. coli* isolates, 22(28.95%) were found to be ESBL producing and 54(71.05%) were ESBL non-producing. Out of 22 ESBL producing, 18(81.81%) were isolated from urine, 3(13.64%) from sputum, and 1(4.55%) from pus.

Out of 76 *E. coli* isolates, 9(11.84%) were ESBL producer isolated from male and 13(17.10%) were ESBL producer isolated from female. Similarly, 19(25.00%) were ESBL non-producer isolated from male and 35(46.05%) were ESBL non-producer isolated from female. Among 22 ESBL producer, a high frequency of 4(18.18%) was isolated from female of age group 20-29 yrs.

The antibiotic susceptibility of ESBL producer showed maximum sensitivity to Colistin i.e. 22(100%) followed by Imipenem 21(95.45%) and meropenem 21(95.45%).

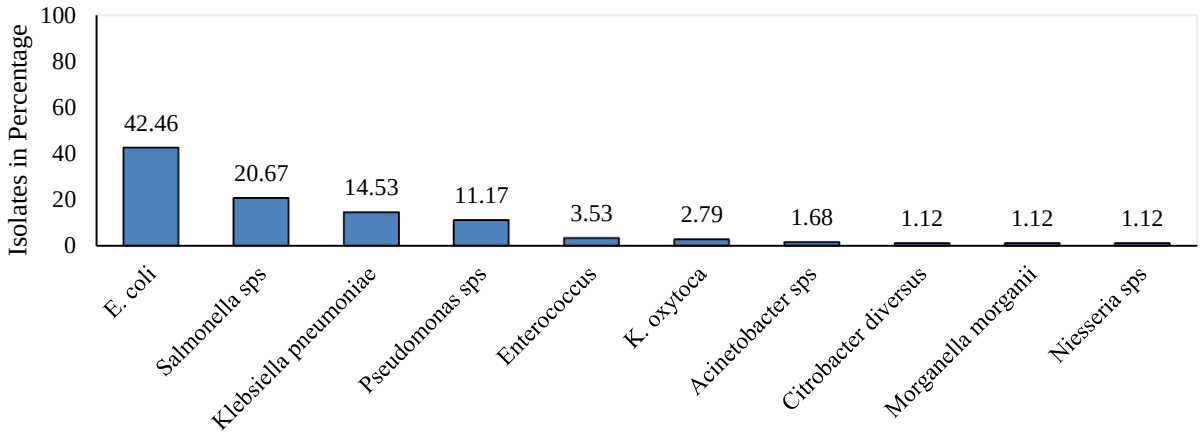


Figure 1: Gram-negative bacteria in clinical specimen

Whereas maximum resistance was towards Amoxycillin 22(100%) followed by Cefixime 20(90.91%). In case of ESBL non-producer, maximum resistance was shown against Amoxycillin 30(55.56%). followed by Ciprofloxacin 30(55.56%).

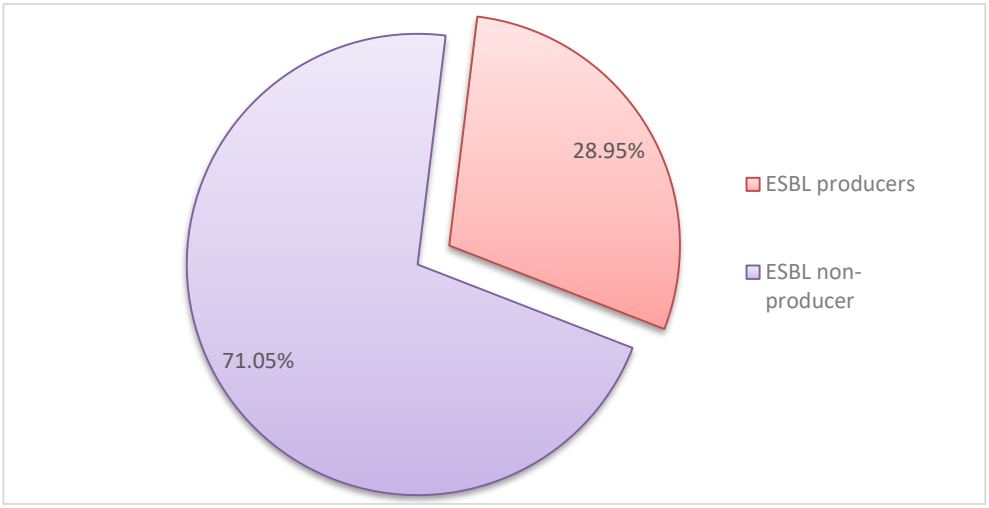


Figure 2: Prevalence of ESBL

Table 1: Distribution of ESBL producing and non-producing E. coli according to clinical specimen

Clinical Specimen	ESBL producer n (%)	ESBL non-producer n (%)	Total n (%)
Blood	-	9(16.67%)	9 (11.84)
Sputum	3 (13.64%)	-	3(3.95)
Pus	1(4.55%)	1(1.85%)	2(2.63)
Urine	18(81.81%)	44(81.48%)	62(81.58)
Total	22(28.95%)	54(71.05%)	76(100%)

Out of 22 ESBL producing *E. coli*, 14(63.64%) was found to be non-biofilm producer, 4(18.18%) was found to be moderate biofilm producer and 4(18.18%) was found to be strong biofilm producer. Likewise, among 54 ESBL non-producers 41(75.93%) was found to be non-biofilm producer, 11(30.37%) was found to be moderate biofilm producer and 2(3.70%) was found to be strong biofilm producer.

Hence, among 22 ESBL producer, 8(36.36%) was found to be biofilm producer whereas among 54 ESBL non-producer 13(24.07%) was found to be biofilm producer. There was statistically no significant association (p=0.105) between biofilm formation capacity and *E. coli*.

Table 2: Age and Gender wise distribution of ESBL producing and non-producing *E. coli*

Age of patients in years	ESBL producer (n=22)		ESBL non-producer (n=54)		Total (%)
	Male (%)	Female (%)	Male (%)	Female (%)	
0-9	-	-	-	-	-
10-19	0	1	2	4	7 (9.20)
20-29	2	4	7	8	21(27.63)
30-39	2	2	2	7	13(17.1)
40-49	2	2	1	1	6(7.89)
50-59	2	3	2	7	14(14.42)
60-69	1	1	3	6	11(14.50)
70 above	0	0	2	2	4(5.26)
Total	9 (11.84)	13(17.10)	19(25.00)	35(46.05)	76(100%)

Table 3: Antibiotic susceptibility pattern of ESBL producing and non-producing *E. coli*

Antibiotics used	ESBL producer (n=22)		ESBL non-producer (n=54)	
	Sensitive (%)	Resistance (%)	Sensitive (%)	Resistance (%)
Amoxycillin (AMX)	0(0)	22(100)	24(44.44)	30(55.56)
Cefixime (CFM)	2(9.09)	20(90.91)	30(55.56)	24(44.44)
Ceftriaxone (CTR)	0(0)	22(100)	46(85.19)	8(14.81)
Ciprofloxacin (CIP)	5(22.73)	17(77.27)	24(44.44)	30(55.56)
Cotrimoxazole (COT)	7(31.82)	15(68.18)	34(62.96)	20(37.04)
Gentamicin (GEN)	15(68.18)	7(31.82)	48(88.89)	6(11.11)
Imipenem (IMP)	21(95.45)	1(4.55)	54(100)	0(0)
Meropenem (MRP)	21(95.45)	1(4.55)	54(100)	0(0)
Nitrofurantoin (NIT)	15(68.18)	7(31.82)	54(100)	0(0)
Polymyxin/CL	22(100)	0(0)	54(100)	0(0)

Table 4: Biofilm production by *E. coli* in Tissue Culture Plate method

E. coli	Strong Biofilm producer	Moderate biofilm producer	non-biofilm producer	Total	p-value
ESBL producer	4(18.18)	4(18.18)	14(63.64)	22(28.95)	0.105
ESBL non-producer	2(3.70)	11(20.37)	41(75.93)	54(71.05)	
Total	6(7.89%)	15(19.74%)	55(72.37%)	76(100%)	

Table 5: Antibiotic susceptibility pattern among biofilm producing and non-producing ESBL producer *E. coli*

Antibiotics used	Biofilm producer (n=8)		Biofilm non-producer (n=14)	
	Sensitive (%)	Resistance (%)	Sensitive (%)	Resistance (%)
Amoxycillin (AMX)	0(0)	8(100)	0(0)	14(100)
Cefixime (CFM)	1(12.5)	7(87.5)	1(7.14)	13(92.86)
Ceftriaxone (CTR)	0(0)	8(100)	0(0)	14(100)
Ciprofloxacin (CIP)	1(12.5)	7(87.5)	4(28.57)	10(71.43)
Cotrimoxazole (COT)	3(37.5)	5(62.5)	4(28.57)	10(71.43)
Gentamicin (GEN)	4(50.0)	4(50.0)	11(78.57)	3(21.43)
Imipenem (IMP)	7(87.5)	1(12.5)	14(100)	0(0)
Meropenem (MRP)	7(87.5)	1(12.5)	14(100)	0(0)
Nitrofurantoin (NIT)	7(87.5)	1(12.5)	8(57.14)	6(42.86)
Polymyxin/CL	8(100)	0(0)	14(100)	0(0)

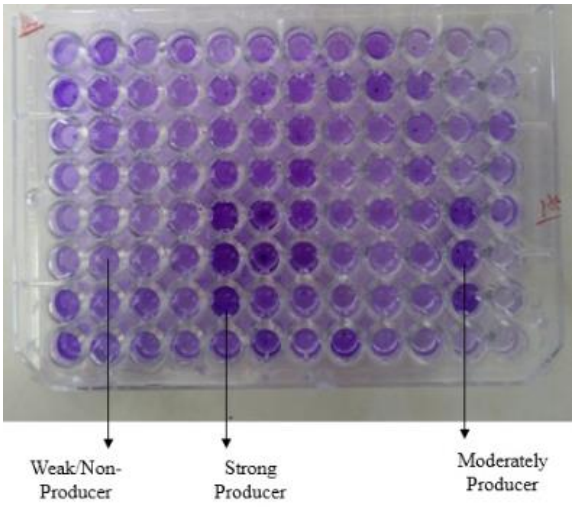
Table 6: Antibiotic susceptibility pattern among biofilm producing and non-producing ESBL non-producer *E. coli*.

Antibiotics used	Biofilm producer (n=13)		Biofilm non-producer (n=41)	
	Sensitive (%)	Resistance (%)	Sensitive (%)	Resistance (%)
Amoxycillin (AMX)	0(0)	13(100)	24(58.54)	17(41.46)
Cefixime (CFM)	7(53.85)	6(46.15)	23(56.09)	18(43.91)
Ceftriaxone (CTR)	10(76.92)	3(23.08)	36(87.80)	5(12.20)
Ciprofloxacin (CIP)	8(61.54)	5(38.46)	18(43.91)	23(56.09)
Cotrimoxazole (COT)	8(61.54)	5(38.46)	26(63.41)	15(36.59)
Gentamicin (GEN)	11(84.62)	2(15.38)	37(90.24)	4(9.76)
Imipenem (IMP)	13(100)	0(0)	41(100)	0(0)
Meropenem (MRP)	13(100)	0(0)	41(100)	0(0)
Nitrofurantoin (NIT)	13(100)	0(0)	41(100)	0(0)
Polymyxin/CL	13(100)	0(0)	41(100)	0(0)

Photograph 1: Lactose fermenting colony of *E. coli* in MA

Photograph 2: Antibiotic susceptibility of *E. coli* **Resistant**: 1. Amoxicillin, 2. Cotrimoxazole, 3. Cefixime, 4. Ceftriaxone; **Sensitive**: 5. Gentamycin, 6. Nitrofurantoin

Photograph 3: ESBL positive *E. coli*
1. CAZ (Ceftazidime); 2. CAC (Ceftazidime+ Clavulanate)



Photograph 4: Biofilm production by Tissue Culture Plate

DISCUSSION

Among 179 gram- negative isolates, 76(42.46%) were found to be *E. coli*. The study conducted by Adhikari et al (2018) showed 68.69% *E. coli,* Acharya et al (2011) reported 68.77% and in the report of Ghorbani et al (2012), *E. coli* occupied 73.5% out of 3000 culture positive isolates. Most of the study showed *E. coli* is responsible for greater frequency of infection. This high frequency might be due to its ubiquitous nature and large number of virulence factors associated with it.

Among 76 *E. coli* isolates, 22(28.95%) were found to be ESBL producing. Similar study conducted by Kayastha et al (2020) reported 28.2% ESBL producer and that of Rimal et al (2017) reported 25.5% of ESBL producing *E. coli*. Prevalence of ESBL producing *E. coli* is variable worldwide. As reported by Batchoun et al (2009) lower prevalence of 2.2% in USA, 2.7% in Canada, 11.4% in China, 9.6% in Saudi Arabia and 11.7% in Kuwait was observed which is low as compared to the present study. Higher prevalence of 62% ESBL was reported by Al-jamei et al (2019). The reason for variable prevalence rate of ESBL producing isolates may be variable affinity of these enzymes for different substrates, inoculums and variable laboratory procedure operated worldwide such as use of updated technology in PCR (Sherchan et al 2015).

In this study, the highest frequency of ESBL producing *E. coli*, 18(81.81%) was obtained from urine followed by 3(13.64%) from sputum and 1(4.55%) from pus. This result can be compared with the result of Sasirekha et al (2010) where 76% of ESBL producing *E. coli* were isolated from urine, 18.7% from sputum and 5.2% from pus. This might be due to higher frequency of *E. coli* was obtained from urine sample and due to the fact that urinary tract infections are among the most common infections encountered in clinical practice (Mohsin et al 2010). *E. coli* can bind to the glycoconjugate receptor of the uroepithelial cells of human urinary tract and initiate infection itself (Jacobsen et al 2008, Eshwarappa et al 2011).

In present study, out of 76 *E. coli* isolates, 9(11.84%) were ESBL producer isolated from male and 13(17.10%) were ESBL producer isolated from female. Similarly, 19(25.00%) were ESBL non-producer isolated from male and 35(46.05%) were ESBL non-producer isolated from female. Among 22 ESBL producer, a high frequency of 4(18.18%) was isolated from female of age group 20-29 years because higher number of *E. coli* was also obtained from the similar age group. Similarly, the study conducted by Shakya et al (2017) reported 27.3% ESBL producing *E. coli* in the age group 21-30 years whereas in the study conducted by Fatima et al (2018), high percentage of 35.7% was found in the age group 46-60 years.

In this study, ESBL producing *E. coli* isolates were significantly more resistant to antibiotics as compared to non-producers of ESBL. The antibiotic susceptibility pattern of ESBL producer showed maximum resistance towards ceftriaxone (100%), Amoxycillin (100%) followed by Cefixime (90.91%), ciprofloxacin (77.27%) and cotrimoxazole (68.18%) whereas these bacteria were found to be sensitive to colistin (100%), imipenem (95.45%),

meropenem (95.45%), nitrofurantoin (68.18%) and gentamicin (68.18%). Similarly, a study conducted by Ndugulile et al (2005) revealed that 11 of the 39(28.2%) of the ESBL producers were found to be resistant to gentamicin with 72.7%, ceftriaxone with 100% and the lowest level of resistance were seen for ciprofloxacin with 45.5%. A study conducted by Umadevi et al (2011) reported that ESBL producing *E. coli* were susceptible to imipenem with susceptibility of 100%. This result also accents with Moyo et al (2010) that reported resistance to Cotrimoxazole (90.7%), ciprofloxacin (46.3%), Amoxicillin (100%) and sensitive to imipenem (94.5%). The study conducted by Baral et al (2012) also reported higher resistant to Cotrimoxazole (86.8%), Amoxicillin (94.1%), Ciprofloxacin (92.6%) and Ceftriaxone (100%). In addition to this, in this study the antibiotic susceptibility pattern of ESBL non-producer showed maximum resistance of 55.56% with both amoxycillin and ciprofloxacin. The need of today's generations is to promote rational use of antibiotics in humans and steps should be taken at all levels to minimize the impact and spread of resistance (Shrivastava et al 2018).

In the present study, among 22 ESBL producer, 8(36.36%) was found to be biofilm producer whereas among 54 ESBL non-producer 13(24.07%) was found to be biofilm producer. The study of Neupane et al (2016) reported 29%, Khatri et al (2017) reported 58.1% and Shrestha et al (2019) reported 54.1% biofilm producing ESBL producer. The rate of biofilm production was high among ESBL producing isolates than in ESBL non-producing isolates. Biofilm producing strains showed relatively high drug resistance as compared to non-biofilm producing isolates. This may be because bacterial biofilms are often associated with long term persistence of organisms that facilitate plasmid exchange and hence enhance the spread of antimicrobial resistance (Dash et al 2018).

Among 8 biofilms producing ESBL producer *E. coli*, the isolates were resistant to various drugs amoxicillin (100%), Ceftriaxone (100%), cefixime (87.5%), ciprofloxacin (87.5%) and cotrimoxazole (62.5%). The isolates were only 50% sensitive to gentamicin. Among 14 biofilm non-producer ESBL producer *E. coli*, the isolates were resistance to amoxicillin (100%), ceftriaxone (100%), cefixime (92.86%). In comparison to biofilm producing, biofilm non-producers ESBL producers were found to be more sensitive towards gentamicin (78.57%). Similarly, in the study conducted by Tadepalli et al (2016), biofilm producing *E. coli* were resistant to Amoxicillin (92%), cefixime (91%), ciprofloxacin (75%), Cotrimoxazole (68%), ceftriaxone (67%) and gentamicin (52%). The biofilm forming and ESBL producing *E. coli* showed high resistance rate to almost all the antimicrobial agents used.

Among 13 biofilms producing ESBL non-producer *E. coli*, the isolates were 100% resistance to amoxicillin and 46.15% to cefixime. The isolates were found to be 100% sensitive to imipenem, meropenem, nitrofurantoin and colistin. Among 41 biofilm non-producing ESBL non-producer, the isolates were 56.09% resistance to ciprofloxacin and 41.46% to amoxicillin whereas the isolates were sensitive to Ceftriaxone (87.80%), gentamicin (90.24%), cotrimoxazole (63.41%) and Cefixime (56.09%). All the isolates of biofilm producing and non-producing ESBL non-producer were found to be 100% sensitive towards imipenem, meropenem, nitrofurantoin and colistin. Similar study conducted by Vuotto et al (2014) and Giamarellou H (2010), 100% sensitive was observed towards colistin. It's the last viable option for multidrug resistant strains either being non-producer or producer of ESBL and biofilm.

The important character of biofilm is their increased tolerance to antimicrobial agents which poses a serious health problem. Microbial biofilms have been associated with a variety of persistent infections which respond poorly to conventional antibiotic therapy that enhances mutation rates and increases spread of antibiotic resistant traits (Subramanian et al 2012). From the above study, it can be interpreted that *E. coli* was sensitive towards Imipenem, Meropenem, Nitrofurantoin and Colistin. A study conducted by Mously et al (2016) concluded high sensitivity 99.8% to Imipenem and meropenem. The increased use of antibiotics can cause the emergence of new resistance strains. So, there is need to have a proper regulatory surveillance of antibiotic prescription and also development of new antibiotic discoveries for the betterment in the health care industry.

The present study provides the prevalence of ESBL and non-ESBL producing *Escherichia coli*, load of biofilm producing organisms and their AST pattern. There are some limitations of the present study, due to lack of resources and time the molecular methods for identification of ESBL strains couldn't be carried out.

CONCLUSION

In this study, *E. coli* was predominant among gram-negative bacteria and frequently isolated in urine sample. This study reveals that prevalence of *E. coli* was found higher in female with age group 20-29 years which was similar in case of ESBL producers. Overuse or misuse of antibiotic has led to increase in resistance of various antibiotic. Most of the isolates of ESBL producing *E. coli* were resistant towards amoxicillin, ceftriaxone, cefixime and ciprofloxacin. For the treatment of ESBL infection, currently carbapenems are the drug of choice. Among the ESBL producers and non-ESBL producers, biofilm production was high 36.36% in ESBL producing isolates. The study also found that biofilm producing bacteria were comparatively resistant than biofilm non-producer to antibiotic as formation of biofilm increases the persistence of infection and level of AMR.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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