

Original Article

Antibiogram of Extended Spectrum Beta-Lactamase and AmpC Beta-Lactamase Producing *Escherichia coli* among the Patients Attending a Selected Tertiary Health Care Hospital

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Received: 19 November 2019 Accepted: 3 July 2020

doi: <https://doi.org/10.3329/jemc.v10i3.59360>

Abstract

Background: The global emergence and spread of Extended Spectrum Beta-Lactamases (ESBLs) and AmpC beta-lactamases (AmpC) producing *Escherichia coli* (*E. coli*) have become a global concern because of inducing resistance toward most of the antimicrobial classes and making the treatment difficult. Hence, this study was aimed to assess the prevalence and antibiotic resistance pattern of ESBL and AmpC beta-lactamases producing *E. coli* in Bangladesh Institute of Health Sciences (BIHS) General Hospital. **Objectives:** To assess the prevalence of ESBL +ve and AmpC +ve producing *E. coli* and to see their antibiotic resistance pattern in BIHS General Hospital. **Materials and Methods:** A total of 675 samples were subjected to aerobic bacteriologic culture in the department of Microbiology, BIHS General Hospital, Mirpur, Dhaka, during the period of 1st July 2016 to 30th June 2017. Specimens were collected from hospitalized and outdoor patients of different age and sex groups. All the isolates were identified by standard microbiological technique and their antibiotic susceptibility was observed by disk diffusion method. Strict aseptic precautions were taken all through the culture system. **Results:** Out of 675 specimens, 150 (22.22%) culture yielded growth of *E. coli*. Among them 47 (31.3%) were ESBL +ve, 27 (18%) were AmpC +ve and 7 (4.7%) were both AmpC +ve and ESBL +ve *E. coli*. **Conclusion:** Advance antibiotic resistance surveillance is necessary to guide the appropriate and judicious antibiotic use. The antibiotic should be used after performing culture and sensitivity test to minimize increasing trend of drug resistance.

Key words: Extended spectrum beta-lactamases; AmpC beta-lactamases; *Escherichia coli*; Antimicrobial resistance

J Enam Med Col 2020; 10(3): 179–185

Introduction

Suffering from several chronic uncontrolled bacterial infections often leads to terminal diseases. It is found that infection by drug-resistant bacteria is the cause of morbidity and mortality.¹ Pathogenic bacteria gain multidrug resistance (MDR) by mutation or

by different processes of transfer of drug resistance. The mutation frequency of antibiotic-resistance is one in 10^6 – 10^8 /cell.² The evolutionary capabilities of a few pathogenic gram-negative (GN) bacteria are so versatile that notorious pan drug resistant (PDR;

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some strains of these bacteria are resistant to almost all contemporary antibiotics) strains have emerged; they are identified mainly as *Escherichia coli* (*E. coli*), *Acinetobacter*, *Pseudomonas* and *Klebsiella pneumoniae*.³ The outbreak of multi-drug resistance bacterial strains and their rapid spread affects the cost of hospitalization and is a great public health concern. Multidrug-resistant Enterobacteriaceae, including *E. coli*, are detected at an increasingly alarming rate throughout the world.⁴ The increasing resistance of *E. coli* is mainly attributed to the pandemic spread of plasmids harboring CTX-M cefotaximases, conferring resistance to both beta-lactam antibiotics and other antibiotic groups, including fluoroquinolones and aminoglycosides.⁵

In addition, many multi-drug resistant *E. coli* are also associated with significant pathogenic potential, causing a wide range of infections.⁶ Together with a rapidly declining pipeline of new antibiotics, this emergence of multi-drug resistant, pathogenic *E. coli* raises serious concerns about patient and public health.⁷ In this context, it is recommended screening for multidrug resistant micro-organisms (MDROs) including *E. coli* should be done from time to time.⁸

Escherichia coli (*E. coli*) is the major aerobic member of the normal intestinal flora. Some strains are able to produce diarrheal disease. *E. coli* is passed in stool and then may contaminate soil, water etc.

Gram negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are among the most important pathogens causing both nosocomial and community-acquired infections.⁹ Among the gram-negative bacteria, *Escherichia coli* causes a wide range of diseases such as diarrhea, urinary tract infection, meningitis and sepsis which can lead to death.¹⁰

Bacterial resistance to beta-lactam antibiotics and emergence of multidrug resistance is a major problem in developed and developing countries.¹¹ Beta-lactamases are the most important mechanism of antimicrobial resistance among gram-negative bacteria.¹²

Multi-drug resistant *E. coli* are widely distributed in hospitals and are increasingly being isolated from community. Thus, it is urgent to find out new

antimicrobial agents.^{13,14,15} However, new families of antimicrobial agents have a short life expectancy.¹⁵ Several monitoring programs have been initiated to generate baseline data about the prevalence of MDR in different bacterial species, including *E. coli*.^{13,16}

This study was aimed to assess the prevalence of ESBL +ve and AmpC +ve producing *E. coli* isolated from the different clinical specimens of the patients attending at a tertiary health care hospital, Dhaka and their antibiotic susceptibility pattern of commonly used antibiotics.

Materials and Methods

The study was carried out in the department of microbiology, BIHS General Hospital during the period from 1st July 2016 to 30th June 2017. The study was designed as descriptive type of cross-sectional study. A total of 675 samples (urine, pus, wound swab, HVS and blood) were collected from the outpatient department and inpatient department of BIHS general hospital by strict aseptic technique. The samples were properly labeled with patient name, ID number etc. Then the specimens were transferred to the laboratory as quickly as possible, usually within 1 hour after collection.

Specimens were inoculated into bacteriological culture media (blood agar, MacConkey agar, nutrient agar etc.) and incubated aerobically at 37^o C for 24 hours. After completion of incubation, the inoculated culture plates were observed for presence of any bacterial growth. Identification of the bacterial isolates were performed on the basis of gram staining, colony characteristic and relevant biochemical tests using standard microbiological methods.

Double disk diffusion test (DDDT) was used as screening test. Disks of cefotaxime (CTX), ceftazidime (CAZ) and amoxycylav (AMC) were placed in a line, placing AMC in the centre and other two on either side. Distance between AMC and other two were 25 mm from centre to centre. Other antibiotic disks were placed as standard procedure in the same plate along with screening test for ESBL activity. Widening of cephalosporin's inhibition zone adjacent to the disk was regarded as ESBL positive.¹⁷

Twenty five antibiotic disks were examined for antimicrobial sensitivity test. When amoxycylav,

ceftazidime and cefotaxime produce resistant in a row in Molar Hinton agar media, usually they produce “Key” shape is called ESBL producing *E. coli*.^{18,19} Similarly, When amoxyclav, ceftazidime, cefotaxime and cefoxitin produce resistant pattern in a row is called AmpC beta-lactamase producing *E. coli*. Extended spectrum beta-lactamase (ESBL+ve) is supposed to be resistant to penicillin, most of cephalosporins and aztreonam. On the other hand, AmpC +ve bacteria are resistant to all penicillin, all cephalosporins (including

cephamycin) and Monobactams.^{20,21}

Results

Age and sex distribution of the study population are shown in the Fig 1. In the present study, a total of 675 patients were included; gender distribution of the study population showed that females are predominant (84%) than males. Among the participants majority (52.90%) were in the age group of 61–70 years and lowest (5%) were in the 0–10 years age group.

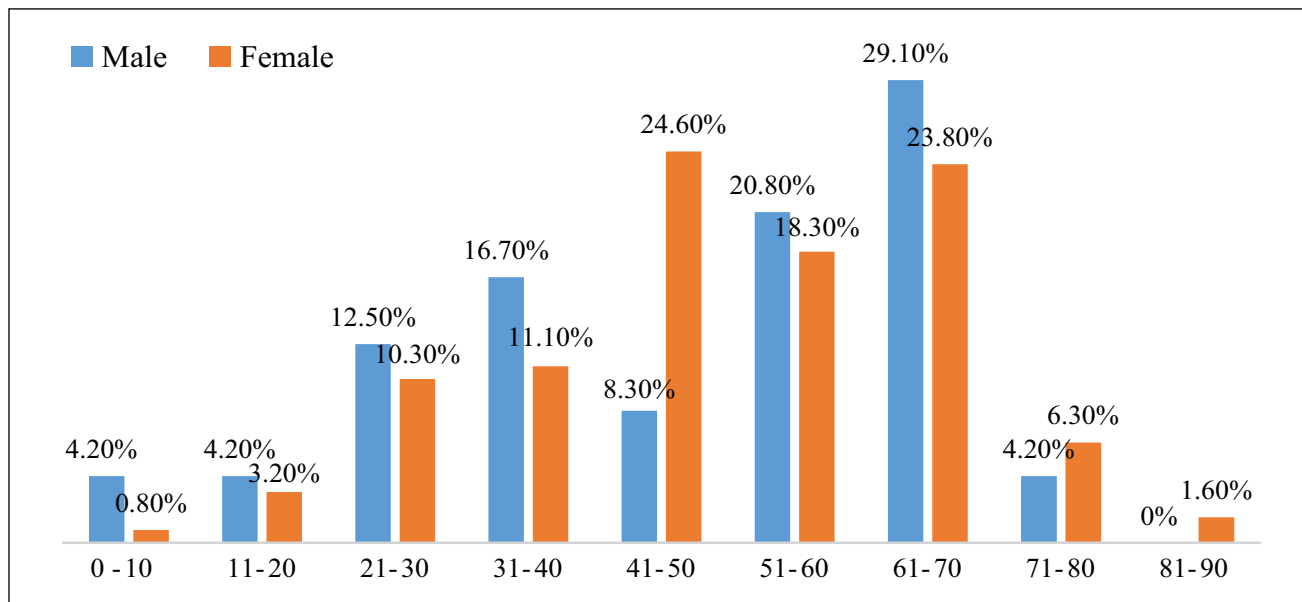


Fig 1. Age (in years) and sex distribution of the study population

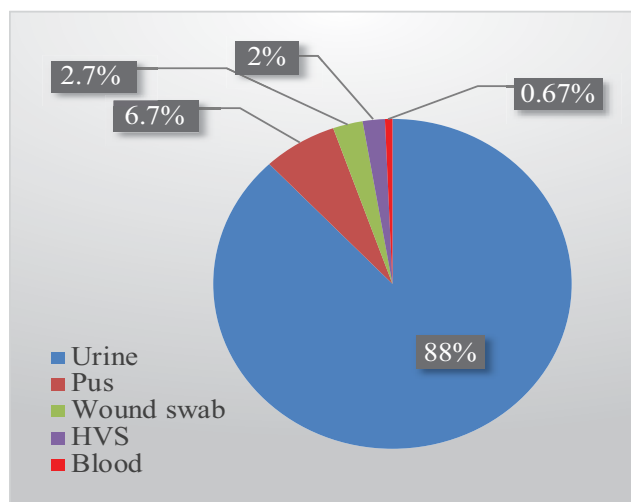


Fig 2. Rate of isolation of *E. coli* from different types of clinical specimens

Table I: Rate of detection of ESBL+ve and AmpC +ve *E. coli* (n=150)

Organisms	Number	Percentage
AmpC +ve	27	18.0
ESBL +ve	47	31.3
Both AmpC +ve & ESBL+ve	7	4.7
Total	81	54.0

Table II: Rate of detection of ESBL+ve and AmpC+ve *E. coli* in different samples

Type of resistance	Urine n=132	Pus n=10	Wound Swab n=4	HVS n=3	Blood n=1	Total n=150
Only ESBL +ve	37 (28.0)	4 (40.0)	4 (100)	1 (33.3)	1 (100)	47 (31.3)
Only ApmC +ve	22 (16.7)	3 (30.0)	0 (0)	2 (66.7)	0 (0)	27 (18.0)
Both ApmC +ve & ESBL +ve	6 (4.5)	1 (10.0)	0 (0)	0 (0)	0 (0)	07 (4.7)
Total	65 (49.2)	8 (80)	4 (100)	3 (100)	1 (100)	81 (54)

Table III: Antimicrobial sensitivity pattern of the ESBL +ve and AmpC +ve *Escherichia coli* (n=81)

Serial	Drugs	Resistant <i>E.coli</i> (n=81)			Non-resistant <i>E.coli</i> (n=69)
		AmpC +ve (n=27)	ESBL +ve (n=47)	Both AmpC +ve & ESBL +ve (n=7)	
1	Amoxyclav	0 (0)	0 (0)	0 (0)	63 (91.3)
2	Cefixime	0 (0)	0 (0)	0 (0)	69 (100)
3	Ceftazidime	0 (0)	0 (0)	0 (0)	66 (95.5)
4	Ceftriaxone	0 (0)	0 (0)	0 (0)	66 (95.5)
5	Cefuroxime	0 (0)	0 (0)	0 (0)	24 (34.7)
6	Cephradine	0 (0)	0 (0)	0 (0)	8 (11.5)
7	Cefotaxime	0 (0)	0 (0)	0 (0)	66 (95.5)
8	Cefepime	0 (0)	0 (0)	0 (0)	66 (95.5)
9	Ampicillin	0 (0)	0 (0)	0 (0)	0 (0)
10	Aztreonam	0 (0)	0 (0)	0 (0)	0 (0)
11	Tetracycline	0 (0)	0 (0)	0 (0)	0 (0)
12	Doxycycline	0 (0)	0 (0)	0 (0)	0 (0)
13	Gentamicin	19 (70.3)	44 (93.6)	5 (71.4)	69 (100)
14	Netilmicin	19 (70.3)	44 (93.6)	5 (71.4)	69 (100)
15	Colistin	27 (100)	47 (100)	7 (100)	69 (100)
16	Imipenem	27 (100)	47 (100)	5 (71.4)	69 (100)
17	Meropenem	27 (100)	47 (100)	5 (71.4)	69 (100)
18	Tigycycline	27 (100)	47 (100)	7 (100)	69 (100)
19	Ciprofloxacin	9 (33)	11 (23.4)	2 (28.6)	44 (63.7)
20	Nitrofurantoin	18 (66.7)	41 (87.2)	3 (42.8)	64 (92.7)
21	Cefoxitin	0 (0)	45 (95.7)	0 (0)	69 (100)
22	Nalidixic Acid	0 (0)	1 (2.1)	0 (0)	2 (2.8)
23	Co-trimoxazole	1 (3.7)	8 (17)	0 (0)	15 (21.7)
24	Amikacin	17 (63.0)	45 (95.7)	5 (71.4)	69 (100)
25	Levofloxacin	6 (22.2)	11 (23.4)	1 (14.9)	41 (59.4)

Rate of isolation of *E. coli* from different types of clinical specimens is shown in Fig 2. Most of the *E. coli* were isolated from urine (88%) followed by pus (6.7%).

Out of 675 specimens cultured, *E. coli* were isolated from 150 (22.22%) samples. Of them 47 (31.3%) were ESBL +ve, 27 (18%) were AmpC +ve and 7 (4.7%) were both AmpC+ve and ESBL +ve (Table I).

Isolation of *E. coli* from various samples

Most (65, 49.2%) of the *E. coli* isolated as ESBL, AmpC beta-lactamase and both positives were from urine followed by pus 8 (80%). However rate of detection resistance pattern of *E. coli* is least in urine isolates (49%) than in others (80–100%).

Twenty six antibiotics were used to see the sensitivity pattern of *E. coli* isolated in 150 cases. Hundred percent (100%) of the ESBL +ve *E. coli* was resistant to cephalosporin group of antibiotics, but about 100% sensitive to tigecycline, gentamicin, netilmicin, imipenem, meropenem, ciprofloxacin, nitrofurantoin and colistin antibiotics. In case of AmpC +ve *E. coli* the scenario is same as ESBL +ve ones. All non-MDR *E. coli* were 90–100% sensitive to cephalosporins (Table III).

Discussion

ESBL or AmpC *beta-lactamases* or both ESBL and AmpC *beta-lactamases* producing organisms are the bacteria that have become resistant to multiple antibiotics, and these antibiotics can no longer be used to control or kill these bacteria.²² Antibiotics are important because they help fight against infections that are caused by bacteria.

In the present study, in 54% of the isolated *E. coli* in which ESBL+ve organisms were predominant and AmpC +ve organisms were less predominant. According to the study of Sarojamma & Ramakrishna²³ the ESBL+ve rate from blood samples was 57.14%. In the study of Saffar et al²⁴ the AmpC+ve rate of *E. coli* was 163/206 (79.13%) in urine, 8/14 (57.14%) in blood. About 31% of the isolated *Escherichia coli* was ESBL +ve in the present study and higher (54.5%) in

the study of Asna et al²⁰.

In this study, 18% of *Escherichia coli* was AmpC beta-lactamases +ve. Findings were much lower (7.14%) in the study of Laghawe et al²⁵ and much higher (66.43%) in the study of Rodriguez et al²⁶. Co-expression of AmpC beta-lactamases and ESBLs in single isolate were found in 4.7% cases in the present study. This is similar to the findings (4.86%) in the study of Laghawe et al²⁵. However, the proportion was slightly higher (9.9%) in the study of Avasthi et al²¹. The figure is 19.4% in the study of Kharat et al²⁷. Co-existence of different classes of beta lactamases in a single bacterial isolate poses a challenge both in diagnosis and therapy.²¹ Cefoxitin disk was used for screening AmpC β -lactamase. However, confirmatory test for AmpC β -lactamase was not done in this study. In the study of Avasthi et al²¹ screening for AmpC β -lactamase was done by cefoxitin and confirmed by E test. Among the screening +ve ones 28% (n= 39) was found to be AmpC beta-lactamase negative by confirmatory test. These drug resistances as shown by screening +ve but AmpC beta-lactamase negative by confirmatory test may be by other mechanisms such as decreased permeability of porins or increased efflux pump for these drugs. Ananthan & Subha²⁸ showed loss of porins in 50% isolates of cefoxitin resistant *Escherichia coli*. So, confirmatory test for AmpC beta-lactamase is not important in terms of outcome of drug resistance and therapeutic purpose; however, it is important in academic and epidemiological purpose.

ESBL and AmpC beta-lactamase positive strains were 100% resistant to 1st, 2nd and 3rd generation cephalosporin and aztreonam (exception is that ESBL producing organisms are sensitive but AmpC beta-lactamase producing organisms are resistant to cefoxitin). The ESBL and AmpC β lactamase negative strains were 90–100% sensitive to cephalosporins. The difference was statistically significant ($p < 0.05$). This is also evidenced in the study of Avasthi et al²¹.

Other than ampicillins, cephalosporins and aztreonam, the AmpC beta-lactamase +ve organisms showed varying degrees of resistance to ciprofloxacin

(67%), co-trimoxazole (95.3%), gentamycin (29.7%), netilmycin (29.7%), nalidixic acid (100%), nitrofurantoin (33.7%), tetracycline (100%) whereas the AmpC beta-lactamase negative organisms shows less resistance to these drugs which in most cases is statistically significant ($p < 0.05$). The scenario is almost same in case of ESBL+ve organisms. This correlates with findings of Avasthi et al²¹, Asna et al²⁰ and Yasmin²⁹.

However, all the organisms either ESBL or AmpC beta-lactamase positive or negative are highly (70–100)% sensitive to colistin, meropenem and tigecycline. This is similar to the findings of Avasthi et al²¹, Asna et al²⁰ and Yasmin²⁹. For identification of ESBL producing organisms, in present study double disc diffusion (DDD) test has been used in primary culture plate. CLSI suggested suspicion of ESBL if zone of inhibition of cephalosporin is < 20 mm, which is to be confirmed later by DDD or other tests. Asna et al¹⁷ suggested use of DDD test in routine sensitivity plate which saves 24 hours. Most of the *E. coli* were isolated from urine (88%) followed by pus (6.7%). However, proportion of AmpC +ve in *E. coli* is least in urine isolates (79.13%) which is great proportion than in others, for example *Klebsiella spp* (33/206, 16.02%) and *P. vulgaris* (10/206, 4.85%).³⁰ This is consistent with the study of Ibrahim et al³⁰ where urine sample was 65.1% followed by wound swab (22.0%). The proportion of females were more (84%, $n=150$) than males. The difference is statistically significant ($p < 0.05$). In the study of Ibrahim et al³⁰ also females were predominant (59.5%, $n=232$). Age group 61–70 years were predominating (24.7%) followed by age group 41–50 years (22.0%). However, Ibrahim et al³⁰ divided the study population into adults and children. The proportion of adults were more (78.5%) than children.

In BIHS General Hospital, Mirpur, Dhaka, *Escherichia coli* is the leading cause of some alarming diseases. It is quite alarming to note that most of the isolates included in this study were found resistant to most commonly used antibiotics (ampicillin, cephalosporins, ciprofloxacin and tetracycline). But carbapenems, colistin, tigecycline and netilmycin are still highly effective against these multi-drug resistant

Escherichia coli. However, drugs should be prescribed according to culture and sensitivity report.

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