**Journal of Monno Medical College**
June, 2025; 11(1):49-53

*Original Article*

# Detection of Extended Spectrum Beta-lactamase (ESBL) Producing Gram Negative Bacteria from Different Clinical Specimens of Largest Teaching Hospital in Dhaka City of Bangladesh.

Bhuiyan Mohammad Mahtab Uddin[1], Mosfika Mahjabin[2], Md. Anisur Rahman[3], Zubair Ahmed Ratan[4], Mohammad Nahid Salman[5], Maruf Ibne Monowar[6], Md. Faizur Rahman[7]

*[1]Associate Professor, Department of Microbiology, Enam Medical College, Dhaka, Bangladesh; [2]Assistant Professor, Department of Pharmacology, Enam Medical College, Dhaka, Bangladesh; [3]Assistant Professor, Department of Microbiology, Noakhali Medical College, Noakhali, Bangladesh; [4]Lecturer and Project Coordinator, School of Health and Society University of Wollongong Wollongong, Australia; [5]Associate Professor, Department of Physiology, Ad-din Akij Medical College, Khulna, Bangladesh; [6]Associate Professor, Department of Anatomy, Enam Medical College, Dhaka, Bangladesh; [7]Lecturer, Department of Microbiology, Dhaka Medical College, Dhaka, Bangladesh.*

**Abstract**

**Background*:*** Detection of Extended spectrum beta lactamase (ESBL) enzyme producing bacteria in hospital settings is very important as ESBL genes are transmissible. **Objective:** This study was carried out to determine the distribution of ESBL producing gram negative isolates at a tertiary care hospital in Dhaka city which deals with different types of patients. **Methodology:** Clinical specimens were collected from the patients attending the microbiology laboratory of Dhaka Medical College from outpatient and inpatient department, Dhaka, during the period of July 2014 to June 2015. **Results:** Out of 191-gram negative bacteria 73 (38.22%) were positive for ESBL production by DDS test. Those out of 73 ESBL producers 50 (68.49%) were positive for ESBL encoding gene *bla*CTX-M-15 and 36 (49.32%) for *bla*OXA-1 by PCR. By DDS test, Among the ESBL producers, *Escherichia coli* was the highest (43.84%%) which was followed by *Pseudomonas* species (15.07%), *Klebsiella species* (15.07%), *Citrobacter spp*. (8.22%) and *Proteus species* (6.85%). Out of 24 *Esch. coli* isolated from outpatient department, 7(29.2%) were positive for ESBL. On the other hand, out of 30 Esch coli isolated from inpatient department, 25 (83.33%) were positive for ESBL. The difference was statistically significant ($p<0.001$). **Conclusion:** In conclusion, the distribution of ESBL producers is more among the hospitalized patients than the patients of the community.

**Keywords:** Bangladesh, Dhaka Medical College Hospital, ESBL, Gram negative bacteria
**Received:** 02 April 2025; **Accepted:** 20 May 2025; **Published:** 1 June 2025
**DOI:** https://doi.org/10.3329/jmomc.v11i1.82380

**Correspondence:** Dr. Bhuiyan Mohammad Mahtab Uddin, Associate Professor, Department of Microbiology, Enam Medical College, Dhaka, Bangladesh; email: mahtab.sbmc@gmail.com, **Orchid ID:** 0000-0002-5109-9851.Contact number- 01682780492.

**How to cite this article:** Uddin BMM, Mahjabin M, Rahman MA, Ratan ZA, Salman MN , Monowar MI, Rahman MF. Detection of Extended Spectrum Beta-lactamase (ESBL) Producing Gram Negative Bacteria from Different Clinical Specimens of Largest Teaching Hospital in Dhaka City of Bangladesh. J Monno Med Coll. 2025 June;11(1):49-53.

**Copyright:** ©Uddin et al. 2025. This article is published under the Creative Commons CC BY-NC License (https://creativecommons.org/licenses/by-nc/4.0/).

## Introduction

Gram negative bacteria intrinsically can produce both chromosomal and plasmid mediated beta lactamases enzymes due to selective pressure created by beta lactam substances produced by soil organisms. TEM-1 was the 1st plasmid mediated beta lactamase enzyme described in early 1960. Subsequently extended spectrum beta lactamases (ESBLs) are identified[1]. ESBLs are 2be group enzymes of Bush-Jacoby-Medeiros classification and some of group 2d enzymes which has similar functional properties like group 2be enzymes[2]. These enzymes are produced by members of Enterobacteriaceae such as *Esch. coli*, *K. pneumoniae, Citrobacter* spp, *Proteus* spp, *Enterobacter spp, Morganella morganii, Serratia marsescens* and other gram-negative bacteria such as *Pseudomonas aeruginosa* and A*cinetobacter baumannii1*.

ESBLs are efficiently capable of hydrolyzing penicillins, early cephalosporins such as cephaloridine and cephalothin except cephamycins, the oxyimino group containing cephalosporins like cefotaxime, ceftazidime, and monobactam and are usually inhibited by beta lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam[3,5].

In addition, ESBLs genes are frequently intermingled with other antibiotic resistance genes such as tetracycline, aminoglycosides, trimethoprim, sulphonamide, chloramphenicol and quinolones making them multidrug resistance[4]. High prevalence of ESBL producers are documented from all over the country. Prevalence of ESBLs differs significantly geographically and depends on various factors6.Enterobacteriaceae are the most common group of gram-negative rods isolated in clinical laboratories[7]. Hence Detection of ESBL production by Enterobacteriaceae and other gram-negative bacteria have paramount importance to ensure appropriate antibiotic treatment. With this view this study was designed to find out the distribution of ESBL producing bacteria isolated from different clinical specimens of Dhaka Medical College and Hospital in Dhaka city of Bangladesh.

## Methodology

**Study Settings and Population:** This cross-sectional study was conducted in the Departmetn of Microbiology at Dhaka Medical College, Dhaka, Bangladesh from July 2014 June 2015 for a period of one year. The clinical specimens were collected from the patients attending the microbiology laboratory of Dhaka Medical College, Dhaka, Bangladesh from outpatient and inpatient department. In total 191-gram negative bacteria were isolated and identified from different types of samples such as urine, wound swab, endotracheal aspirate and blood. Samples were collected by aseptic standard procedures.

**Detection of Bacterial Species:** Blood agar and MacConkeys's agar media were used for the primary isolation of the bacteria. Identification of every particular gram-negative bacterium was done by Gram staining, observing colony morphology, oxidase test, inoculation into Triple sugar iron (TSI), Motility indole urea (MIU) and Simmons citrate agar media. ESBL producing bacteria was detected by screening test that is Double disc synergy test (DDST). Then detection of ESBL encoding genes such as *bla*CTX-M-15 and blaOXA-1 were detected by PCR. PCR was taken as gold standard. *Klebsiella pneumoniae* ATCC 700603 was used as reference strain for ESBL positive . control. The strain of *Esch. Coli*, which was sensitive to ceftazidime, ceftriaxone, cefotaxime and aztreonam was used as negative control.

**Screening Test:** Standard inoculum of bacterial suspension matched to 0.5 McFarland was made and Mueller Hinton Agar (MHA) plate was inoculated properly with bacterial suspension. Ceftazidime (30 g), Ceftriaxone (30µg), Cefotaxime (30µg) and Aztreonam (30µg) discs (Oxoid, England) were placed onto MHA plate and incubated overnight at 37ºC. When inhibition zone of any isolate to Ceftazidime ≤22mm or Aztreonam ≤27mm, or Cefotaxime ≤27mm or Ceftriaxone ≤25mm alone or in combination was found then the isolate was taken as screening test positive.

**Double Disc Synergy Test (DDST) test[8-9]:** The MHA plate was inoculated with bacterial suspension matched to 0.5 McFarland. Ceftazidime (30µg), Ceftriaxone (30µg), Cefotaxime (30µg) and Aztreonam (30µg) discs were placed 15 mm distance centre to centre from amoxiclav disc (20mg amoxicillin and 10mg of clavulanic acid) which was placed at middle. Any extension of inhibition zone of antimicrobial discs (one or more) towards amoxiclav disc confirmed the presence of ESBL.

**Molecular Characterization ESBL Producers by PCR[10] test:** The presence of ESBL genes such as *bla*CTX-M-15 and *bla*OXA-1 genes among the ESBL producers were detected by polymerase chain reaction (PCR). To prepare bacterial pellets, a loop full of bacterial colonies was inoculated into a Falcon tube containing trypticase soy broth. After incubation overnight at 37ºC, the Falcon tubes were centrifuged at 4000 ×g for 10 minutes, after which the supernatant was discarded. A small amount of sterile trypticase soy broth was added into the Falcon tubes with pellets and mixed evenly. Then an equal amount of bacterial suspension was placed into 2 to 3 to microcentrifuge tubes. The microcentrifuge tubes were then centrifuged at 4000×g for 10 minutes and the supernatant was discarded. The microcentrifuge tubes containing bacterial pellets were kept at -20ºC until DNA extraction. Bacterial DNA was extracted by the boiling method[7]. The following pairs of previously used primers were used to yield PCR products: for b1aCTX-M-15-CACACGTGGAATTTAGGGACT (forward), GCCGTCTAAGGCGATAAACA (reverse) and for blaOXA-1- ACCAGATTCCAACTTTCAA (forward), TCTTGGCTTTTATGCTTG (reverse)[8]. The following cycling parameters were used: initial denaturation at 95ºC for 10 minutes, then 30 cycles of denaturation at 95º C for

one minute, annealing at 63ºC (for blaNDM-1), 52º C (for *bla*IMP), 52º C (for blaVIM) for 45 seconds, extension at 72ºC for one minute and 30 seconds, and a final extension at 72º C for 10 minutes. The amplified DNA were loaded into a 2% agarose gel, electrophoresed at 100 volts for 30 minutes, stained with 1% ethidium bromide, and visualized under UV light.

**Statistical Analysis:** Statistical analysis was performed by Windows based software named as Statistical Package for Social Science (SPSS), versions 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Continuous data were expressed as mean, standard deviation, minimum and maximum. Categorical data were summarized in terms of frequency counts and percentages. Every efforts were made to obtain missing data.

**Ethical Consideration:** All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration 2013) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by the local ethics committee. Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and were analyzed using the coding system.

## Results

Total isolated gram-negative bacteria were 191 and the most frequently isolated bacteria was Escherichia coli. Out of 54 Escherichia coli, 32 (4.24%) was confirmed as ESBL producers by PCR test (Figure I).

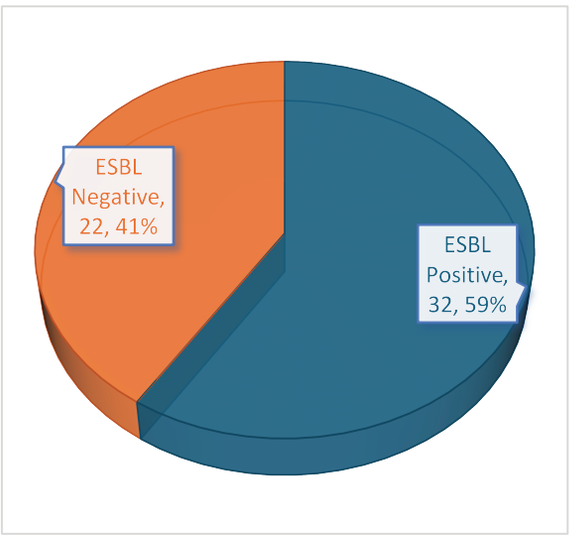


**Figure I: Showing the ESBL Producing Escherichia coli (n=54)**

Significantly highest (83.33%) percentage of ESBL producing *Escherichia coli* had been identified from inpatient department (IPD) and a much less amount of 29.2% isolates. ESBL producing *Escherichia coli* was detected from outpatient department (OPD). Urine was the most common sample from which *Escherichia coli* was isolated. Sixty six percent of total *Escherichia coli* was isolated from urine samples. Out of 24 *Escherichia coli* isolated from urine samples of inpatient department, 21(87.5%) were ESBL producers and 5(41.7%) were ESBL producers among 12 Escherichia coli isolated from urine of outpatient department. Difference of ESBL production by *Escherichia coli* between inpatient and outpatient department was statistically significant (Table 1).

**Table 1: ESBL producing *Escherichia coli* detected by Different Methods**

| Test | Inpatient Department | | | Outpatient Department | | |
|---|---|---|---|---|---|---|
| | ESBL | | Total | ESBL | | Total |
| | Positive | Negative | | Positive | Negative | |
| Screening test | 32 | 04 | 36 | 07 | 11 | 18 |
| DDST | 20 | 16 | 03 | 03 | 15 | 18 |
| PCR | 27 ESBL (27.0%) | 09 | 05 | 05 ESBL (27.0%) | 13 | 18 |

About 32 (59.26%) ESBL producing *Escherichia coli* out of total 54 *Escherichia coli*, and isolated 25.0% of *Pseudomonas* species, 14.29% of *Acinetobacter baumannii* and 42.31% of *Klebsiella* species were ESBL producers. Next to *Escherichia coli*, *Pseudomonas* species numbered second, *Acinetobacter baumannii* numbered third and *Klebsiella* species numbered fourth position among 191 bacterial isolations. ESBL producing *Pseudomonas* species were 11(25.0%) out of 44, ESBL producing *Acinetobacter baumannii* were 4(14.3%) among 28, ESBL producing *Klebsiella* species were 11(42.31%) out of 26, ESBL producing *Citrobacter* species were 6 (40.0%) among 15 and ESBL producing Proteus species were 5(38.46%) out of 13. In all strains number of inpatient ESBL producing isolates were higher than outpatient department (Table 2).

| Bacteria Name | Inpatient Department | | | Outpatient Department | | | ESBL producers out of total respective organisms |
|---|---|---|---|---|---|---|---|
| | ESBL | | Total | ESBL | | Total | |
| | Positive | Negative | | Positive | Negative | | |
| *Esch. Coli* | 27 | 09 | 36 | 5 | 13 | 18 | 32 (59.25%) |
| *Pseudomonas spp.* | 9 | 21 | 30 | 2 | 12 | 14 | 11 (25%) |
| *Klebsiella spp.* | 8 | 10 | 18 | 3 | 5 | 8 | 11 (42.31%) |
| *Citrobacter spp.* | 5 | 7 | 12 | 1 | 2 | 3 | 6 (40%) |
| *Proteus spp.* | 4 | 6 | 10 | 1 | 2 | 3 | 5 (38.46%) |
| *Acinetobacter spp.* | 4 | 22 | 26 | 0 | 2 | 2 | 4 (14.28%) |

## Discussion

Over the years beta lactamase antibiotics are prescribed for both hospital acquired and community acquired infections. The continued use of these antibiotics produces selective pressure for pathogenic and commensal bacteria to produce and maintain beta lactam antibiotic destroying mechanisms. Discovery of different types of beta lactamase enzymes are the best example of this long-continued pressure. Now a days multiple broad-spectrum beta lactamases produced by multi drug-resistant *Klebsiella pneumoniae*, *Acinetobacter* species, *Pseudomonas* aeruginosa and *Enterobacter* species have disseminated through gram-negative pathogens[11].

In this research work ESBL producing isolates were identified from clinical specimens of outpatient (OPD) and inpatient department (IPD). ESBL strains obtained from outpatient department figured out community involvement. In contrast ESBL infections of inpatient department denoted nosocomial participation. This study reported 32(54.2%) ESBL *Escherichia coli* out of total 59 *Escherichia coli*, in which 81.5% was from inpatient department and 31.25% from the outpatient department. Significant presence of higher percentage of ESBL producing *Escherichia coli* in inpatient department in present study indicates certain degree of nosocomial spread of infections. Significantly higher number of ESBL producing Escherichia coli was detected from urine of inpatient department which is consistent with findings of several studies[6,12,13]. Isolated 43.75% of *Enterobacter* species, 54.55% of *Proteus* species and 57.1% of *Klebsiella* species were ESBL producers. Vinodini et al., found low percentage of ESBL Enterobacter species and *Proteus* species and Rao et al., showed higher percentage of ESBL *Proteus* species[14,15].

ESBL *Enterobacter* species, ESBL Proteus species and ESBL *Klebsiella* species isolation numbers were high in IPD than OPD. Identification of ESBL *Acinetobacter* species was very important because this is one of the multidrug resistant pathogens[11] and now a day it is being isolated from various biological specimens. This study also documented ESBL *Pseudomonas* species from IPD samples as reported by other study[15]. Isolations of ESBL *Acinetobacter* (7.24%) and ESBL *Pseudomonas* strains (1.45%) were alarming because they are environmental bacteria, difficult to control[9]. All isolated *Serratia* species were identified from IPD blood samples sent for blood culture and all of them were ESBL producers. *Serratia* infections are clearly related to hospitalization[9]. Comparable findings wer e documented by other studies[16-21].

## Conclusion

The present study reveals significant number of ESBL producing gram negative bacteria which demands routine practice of ESBL testing in microbiology laboratory of Dhaka Medical College hospital for reporting.

**Acknowledgement:** None

**Contributions to authors:** Bhuiyan Mohammad Mahtab Uddin, Mosfika Mahjabin, Md. Anisur Rahman were involved in conceptualization, data collection and literature collection. Data analysis and manuscript writing were done by Zubair Ahmed Ratan & data are analyzed by Mohammad Nahid Salman, Maruf Ibne Monowar. Manuscript revision was done by Md. Faizur Rahman.

**Funding:** This research project was not funded by any group or institution.

**Conflict of Interest:** We do not have any conflict of interest (financial or others).

## References

1. Balan K. Detection of extended spectrum β-lactamase among Gram negative clinical isolates from a tertiary care hospital in South India. Int J Res Med Sci. 2013;1:28-30

2. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrobial Agents and Chemotherapy. 1995;39(6):1211-33.

3. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clinical microbiology reviews. 2005;18(4):657-86

4. Bourjilat F, Bouchrif B, Dersi N, Claude JD, Amarouch H, Timinouni M. Emergence of extended-spectrum beta-lactamases-producing *Escherichia coli* in community-acquired urinary infections in Casablanca, Morocco.

The Journal of Infection in Developing Countries. 2011;5(12):850-5

5. Bush K, Jacoby GA. Updated functional classification of β-lactamases. Antimicrobial Agents and Chemotherapy. 2010;54(3):969-76

6. Kashyap G, Gupta S, Mamoria VP, Durlabhji P, Jain D. Increasing prevalence of extended spectrum beta lactamases (ESBLs) producing E. coli and Klebsiella species in outpatient departments (OPDs) patients in urinary tract infections (UTIs) in tertiary care hospital. International Journal of Current Research and Review. 2013;5(11):80.

7. Brooks GF, Butel JS, Morse SA. Jawetz, Melnick, & Adelberg's Medical Microbiology. (No Title). 2004.

8. Dhillon RH, Clark J. ESBLs: a clear and present danger? Critical care research and practice. 2012;2012(1):625170.

9. Rahman NM, Lutfor AB, Jhora ST, Yasmin M, Haq JA. Detection of CTX-M gene in extended spectrum beta lactamase (ESBL) producing Escherichia coli and Klebsiella species of different hospitals. Bangladesh Journal of Medical Microbiology. 2010;4(2):28-31

10. Franco MR, Caiaffa-Filho HH, Burattini MN, Rossi F. Metallo-beta-lactamases among imipenem-resistant Pseudomonas aeruginosa in a Brazilian university hospital. Clinics. 2010 1;65(9):825-9.

11. Bush K. Bench-to-bedside review: the role of β-lactamases in antibiotic-resistant Gram-negative infections. Critical Care. 2010;14:1-8.

12. Pitout JD, Laupland KB. Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. The Lancet Infectious Diseases 2008 1;8(3):159-66.

13. Auer S, Wojna A, Hell M. ESBL Producing Escherichia coli in Ambulatory Urinary Tract Infections—Oral Treatment Options. Antimicrobial Agents Chemotherapy. 2010;54:4006-8

14. Rahman M, Rahman MM, Jahan WA. Clinical laboratory and molecular detection of extended spectrum beta lactamases: a review update. Bangladesh Journal of Infectious Diseases. 2014;1(1):12-7

15. Rao PS, Basavarajappa KG, Krishna GL. Detection of extended spectrum beta-lactamase from clinical isolates in Davangere. Indian Journal of pathology and Microbiology. 2008 1;51(4):497-9.

16. Vinodhini R, Moorthy K, Palanivel P, Punitha TH, Saranya S, Bhuvaneshwari M, Kanimozhi C. Detection and antimicrobial susceptibility pattern of ESBL producing Gram negative bacteria. Asian J Pharm Clin Res. 2014;7(1):243-7.

17. Altun S, Tufan ZK, Yağcı S, Önde U, Bulut C, Kiniki S. Extended spectrum beta-lactamases, AmpC and metallo beta-lactamases in emerging multi-drug-resistant Gram-negativebacteria in intensive care unit. Sci rep. 2013;2(4):707.

18. Jain A, Roy I, Gupta MK, Kumar M, Agarwal SK. Prevalence of extended-spectrum β-lactamase-producing Gram-negative bacteria in septicaemic neonates in a tertiary care hospital. Journal of Medical Microbiology. 2003;52(5):421-5

19. Islam MS, Yusuf MA, Begum SA, Sattar AA, Hossain A, Roy S. Extended-spectrum-beta-lactamase producing uropathogenic Escherichia coli infection in Dhaka, Bangladesh. African Journal of Bacteriology Research. 2015;7(1):1-7

20. Rahman M, Sultana H, Mosawuir MA, Akhter L, Yusuf MA. Status of Extended Spectrum Beta-Lactamase (ESBL) Producing bacteria isolated from surgical and burn wound at tertiary care hospital in Dhaka City. Bangladesh Journal of Infectious Diseases. 2018;5(1):21-6

21. Jena J, Debata N, Subudhi E. Prevalence of extended-spectrum-beta-lactamase and metallo-beta-lactamase producing multi drug resistance gram-negative bacteria from urinary isolates. Indian Journal of Medical Microbiology. 2013 1;31(4):420.