

Original Article



Prevalence and Antibigram of Extended-Spectrum Beta-Lactamase Producing Gram-negative Bacteria Isolated from Septicemic Neonates in a Tertiary Care Hospital

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Abstract

Background: In recent years, Extended-spectrum beta-lactamase (ESBL) producing microorganisms have complicated treatment of infections due to resistance of ESBL producing strains to a wide range of antimicrobials. **Objective:** Target of this study was to determine the prevalence of ESBL producing gram-negative bacteria in neonatal sepsis cases and to reveal the antimicrobial susceptibility pattern of those isolated ESBL producers. **Materials and Methods:** This cross sectional study was carried out in Dhaka Medical College Hospital (DMCH) over a period of 12 months from January to December in 2016. Following isolation and identification of gram-negative bacteria from blood samples of suspected septicemic neonates, antimicrobial susceptibility test was performed by Kirby Bauer disk-diffusion method and ESBL producers were detected by Double Disk Synergy (DDS) test. **Results:** Among 52 Gram-negative bacteria isolated from 106 blood samples, 34.61% ESBL producers were detected and *Enterobacter* spp. (45%) was predominant followed by *Klebsiella pneumoniae* (33.33%). None of the ESBL producers was resistant to colistin and tigecycline. All ESBL producing *Acinetobacter baumannii*, 77.78% and 66.67% of ESBL producing *Enterobacter* spp and *Klebsiella* spp. respectively showed resistance to meropenem. All ESBL producers were resistant to piperacillin-tazobactam. **Conclusion:** Appropriate measures should be taken to prevent the spread of ESBL producing strains by combining strategies for infection prevention, control and rational use of antibiotics.

Key words: Antibigram, Bangladesh, ESBL.

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Introduction

Recently, management of infections caused by gram-negative bacteria has become an international concern due to increasing emergence of extended-spectrum beta-lactamases (ESBLs).^{1,2} ESBLs producing microorganisms confer resistance to penicillin, first, second and third generation cephalosporins and aztreonam (but not cephamycin and carbapenem) and are inhibited by clavulanic acid.³ ESBL producers were isolated for the first time in Western Europe in mid-1980s and the incidence has been rising since then.⁴

The prevalence and distribution of ESBL producers differ from country to country and from hospital to hospital.⁵ An

undeniably high prevalence rate of ESBL producers in the Asia-Pacific were reported by several surveillance studies despite incidence vary with geographical area and time.^{6,7} A high rate of ESBL producers has been documented by limited number of studies on prevalence of ESBLs in Bangladesh.^{8,9}

ESBLs are present in a variety of enterobacteriaceae and non-enterobacteriaceae including *Pseudomonas* spp., *Acinetobacter* spp.¹⁰ Plasmids encoding ESBL enzymes may carry co-resistance genes for other non-beta-lactam antibiotics also.¹¹ Carbapenems are considered to be the antibiotic of choice in treating infections caused by ESBL producers.^{12,13}

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Materials and Methods

Study design and population

This cross-sectional study was performed in Department of Microbiology, in collaboration with Department of Neonatology, Dhaka Medical College Hospital (DMCH), between the periods of January 2016 to December 2016. Neonates of either sex of age 0-28 days, both inborn and out-born, admitted in Neonatal Intensive Care Unit (NICU) of DMCH with suspected clinical features of sepsis at admission or developed such features afterwards when admitted for other indication, irrespective of antibiotic intake were included. Neonates with congenital anomalies, acute bilirubin encephalopathy, perinatal asphyxia, meconium aspiration syndrome, history of prolong rupture of membrane and prolong labor of mother were excluded from this study. The study was approved by Research Review Committee (RRC) and Ethical Review Committee (ERC) of DMC.

Isolation of gram-negative bacteria

Blood samples were collected with all aseptic precaution and were inoculated into BacT/ALERT PF Plus bottle (bioMerieux, Inc, Durham, North Carolina) and these bottles were processed for automated blood culture using BacT/ALERT 3D 60 Microbial Detection System (bioMerieux, Inc, Durham, North Carolina). Subcultures were done on Blood agar and MacConkey agar media. Isolates were identified by colony morphology, hemolytic criteria, pigment production, different biochemical reactions and staining character as per standard techniques.¹⁴

Antimicrobial susceptibility testing and screening of ESBL producers

Antimicrobial susceptibility pattern was determined by Kirby Bauer disk-diffusion method on Muller-Hinton agar media using commercially available antibiotic disks (Oxoid Ltd, UK) following CLSI, 2015.¹⁵ *Esch. coli* ATCC 25922 was used as control strain. Amoxiclav (amoxicillin 20µg + clavulanic acid 10µg/disk), cefixime (30µg/disk), ceftazidime (30µg/disk), ceftriaxone (30µg/disk), ciprofloxacin (5µg/disk), amikacin (30µg/disk), gentamicin (10µg/disk), meropenem (10µg/disk), colistin sulphate (10µg/disk), piperacillin/tazobactam (100/10µg/disk) antibiotic disks were used. Susceptibility of the gram-negative organism to tigecycline was determined using 15µg tigecycline disk and the criteria of the United States Food and Drug Administration was used for interpretation.¹⁶ Gram-negative isolates those showed resistance to any of ceftriaxone and ceftazidime disks, were considered as suspected ESBL producers.³

Detection of ESBL producers by Double-Disk Synergy (DDS) test

Suspected ESBL producers were further confirmed by DDS test.¹⁷ Amoxiclav disk was placed at the center of the Muller-Hinton agar plate and third generation cephalosporins (ceftriaxone, ceftazidime and cefotaxime) were placed 20mm apart from center of the amoxiclav disk. Inoculated plate was incubated at 37°C for 24 hours. A clear extension of the edge of the inhibition zone of cephalosporin disks towards amoxiclav disk was interpreted as ESBLs production of that isolated gram-negative bacteria (Figure 1).

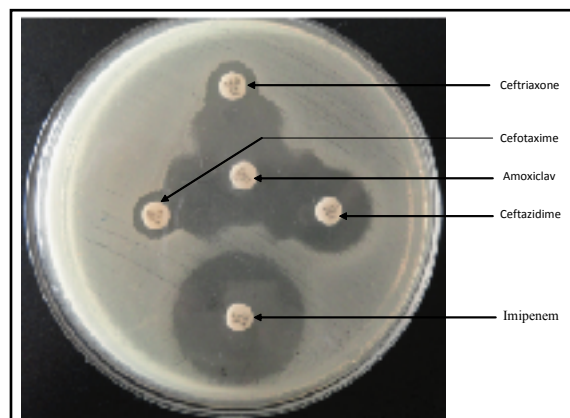


Figure 1: Double-Disk Synergy test for detection of ESBL producers.

Results

Among the isolated 52 gram-negative bacteria, 18 (34.61%) ESBL producers were identified by DDS test. Nine (45%) of the 20 *Enterobacter* spp., 6 (33.33%) of the 18 *Klebsiella pneumoniae*, 2 (25%) of the 8 *Pseudomonas aeruginosa* and one (25%) of the 4 *Acinetobacter baumannii* were ESBL producers (Table I).

Table I: Distribution of ESBL producing gram-negative bacteria identified by DDS test (N=52).

Gram-negative bacteria	ESBL producers by DDS test	
	Positive n (%)	Negative n (%)
<i>Enterobacter</i> spp. (N = 20)	9 (45.00)	11 (55.00)
<i>Klebsiella pneumoniae</i> (N = 18)	6 (33.33)	12 (66.67)
<i>Pseudomonas aeruginosa</i> (N = 8)	2 (25.00)	6 (75.00)
<i>Acinetobacter baumannii</i> (N = 4)	1 (25.00)	3 (75.00)
<i>Citrobacter</i> spp. (N = 2)	0 (0.00)	2 (100.00)
Total (N = 52)	18 (34.61)	34 (65.38)

All the ESBL producing *Enterobacter* spp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *A. baumannii* were resistant to amoxiclav, cefixime, ceftazidime, ceftriaxone, amikacin, gentamicin, piperacillin-tazobactam and were sensitive to colistin, tigecycline. Among the 9 ESBL producing *Enterobacter* spp., 66.67% were resistant to ciprofloxacin and 77.78% to meropenem. Fifty percent of the ESBL producing *Klebsiella pneumoniae* were resistant to ciprofloxacin and 66.67% to meropenem. Among ESBL producing *Pseudomonas aeruginosa*, all were resistant to ciprofloxacin and 50% to meropenem. All the ESBL producing *A. baumannii* were resistant to ciprofloxacin and meropenem (Table II).

Table II: Antimicrobial drug resistance among different species of ESBL producers.

Antimicrobial drugs	Enterobacter spp. (N = 9) n (%)	Klebsiella Pneumoniae (N = 6) n (%)	Pseudomonas aeruginosa (N = 2) n (%)	Acinetobacter baumannii (N = 1) n (%)	Total (N = 18) n (%)
Amoxiclav	9 (100.00)	6 (100.00)	2 (100.00)	1 (100.00)	18 (100.00)
Cefixime	9 (100.00)	6 (100.00)	2 (100.00)	1 (100.00)	18 (100.00)
Ceftazidime	9 (100.00)	6 (100.00)	2 (100.00)	1 (100.00)	18 (100.00)
Ceftriaxone	9 (100.00)	6 (100.00)	2 (100.00)	1 (100.00)	18 (100.00)
Amikacin	9 (100.00)	6 (100.00)	2 (100.00)	1 (100.00)	18 (100.00)
Gentamicin	9 (100.00)	6 (100.00)	2 (100.00)	1 (100.00)	18 (100.00)
Ciprofloxacin	6 (66.67)	3 (50.00)	2 (100.00)	1 (100.00)	12 (66.67)
Meropenem	7 (77.78)	4 (66.67)	1 (50.00)	1 (100.00)	13 (72.22)
Colistin	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Tigecycline	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Piperacillin-Tazobactam	9 (100.00)	6 (100.00)	2 (100.00)	1 (100.00)	18 (100.00)

Discussion

In this study, among 52 gram-negative organisms, 34.61% ESBL producers were detected by DDS test. In agreement with this finding, Rahman¹⁸ reported 31.03% ESBL producers among neonatal sepsis cases. Chelliah et al.¹⁹ from India reported a high proportion of ESBL producers among the neonatal sepsis cases which was 67.3%. The reports from different studies are indicative of the fact that ESBL producers among the neonatal sepsis cases vary greatly geographically and rapidly changing over time. This may be due to irrational, repeated or sub-lethal use of antibiotics. The higher prevalence of ESBL producers in Asia than in Europe and America was observed in a previous study.²⁰ Previous studies in Bangladesh revealed 23% to 31% ESBL producers from Gram-negative bacteria.²¹ However, 80% ESBL producers was also reported in Bangladesh in another study.²² The discrepancy of the findings may be due to the varying prevalence of ESBL producers with time as well as from country to country, city to city and even hospital to hospital in one city.⁸

The prevalence of ESBL producers is increasing in Bangladesh which is reflected by several studies conducted in DMCH irrespective of age and different samples. ESBL producing organisms detected among Gram-negative bacteria from various samples were 29% in 2011 by Islam et al.⁹, 25% in 2012 by Farzana et al.⁸ and 33% in 2015 by Shamsuzzaman.²³

Enterobacter spp. (45%) was the predominant ESBL producer in this study followed by 33.33% of Klebsiella pneumoniae and 25% of Pseudomonas aeruginosa and Acinetobacter baumannii each. Rahman¹⁸ reported 34.62% of Klebsiella Pneumoniae, 75% of Pseudomonas Aeruginosa and 16.67% of Acinetobacter baumannii ESBL producers among neonatal sepsis cases. Katereggea et al.²⁴ reported 25% of ESBL producing Enterobacter cloacae in their study in Uganda. The emergence of ESBL producing Enterobacter spp. and increasing prevalence of ESBL producing Acinetobacter baumannii in neonatal sepsis is clearly evident from this study. However, several studies reported E. coli as most commonly isolated ESBL producers from different clinical samples in Bangladesh.^{8,9,25} The disparity of the isolation rate of different ESBL producers may be due to varying prevalence of infection causing bacteria from one area to area and hospital to

hospital. Different hospital deals with different types of disease and different protocol of antibiotic usage.

In this study, resistance to three or more drugs (multi-drug resistant, MDR) was common in ESBL producers. All the ESBL producers were resistant to ceftriaxone, ceftazidime, cefixim which is similar to the findings reported by Farzana⁸, Rahman¹⁸ and Shamsuzzaman.²³

All the ESBL producers were sensitive to tigecycline and colistin in this study. Rahman¹⁸ reported that 33.33% of the ESBL producers were resistant to colistin where as Shamsuzzaman²³ reported that all the ESBL producers were sensitive to colistin. Canigia and Dowzicky²⁶ reported that 93.7% of the ESBL producing Klebsiella pneumoniae were sensitive to tigecycline which is almost similar to present findings.

All the ESBL producers were resistant to amoxiclav, amikacin, gentamicin and piperacillin-tazobactam which is similar to the findings of the previous study of Rahman.¹⁸ 66.67% of the ESBL producers were resistant to ciprofloxacin. Previously, Rahman¹⁸ and Shamsuzzaman²³ reported that 100% and 95.55% of the ESBL producers were resistant to ciprofloxacin respectively. Kumar et al.²⁷ reported that 60% of the ESBL producing E. coli isolated from blood were resistant to ciprofloxacin. ESBL producing bacteria also resistant to non-B-lactam antibiotics was due to plasmids encoding ESBL genes also carry genes encoding resistance to other agents like aminoglycosides, fluoroquinolones and trimethoprim-sulfamethoxazole.²⁸

72.22% of the ESBL producers revealed resistance to meropenem in this study. Contradictory to this, other studies performed previously in DMCH reported that all of the ESBL producers were sensitive to carbapenem.^{8,19,24} Resistance to carbapenem of ESBL producers might develop spontaneously which is strongly favored by the presence of ESBL encoding plasmid and different mutational spectra. Mainly loss of function mutations in the regulators of porin expression caused reduced influx of antibiotic into the cell and in combination of amplification of B-lactamase genes on the plasmid this led to high resistance level.²⁹

Conclusion

Therapeutic options fall into a narrow range due to emergence of ESBLs in gram-negative bacteria. Reporting of ESBL producers in laboratory should be a routine practice because an excessive occurrences of ESBL producing organisms are evident and these organisms show not worthy resistance to not only beta-lactam antibiotics but also to non-beta lactams. ESBL producing organisms' sensitivity towards carbapenem and piperacillin-tazobactam which are considered as last resource till now has fallen greatly. In this study, colistin and tigecycline revealed remarkable sensitivity but these drugs should be kept as reserved for cases where most other antimicrobials fail to act. Urgent surveillance of ESBL producers by cohort study to acquire an exact prevalence of ESBL producers in our country need to be focused.

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References

1. Paterson DL. Resistance in gram-negative bacteria: Enterobacteriaceae. *American journal of infection control*. 2006; 34(5):S20-28.
2. 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):850-855.
3. Centers for Disease Control and Prevention (CDC). Laboratory detection of extended-spectrum beta-lactamases (ESBLs). Atlanta: CDC, 2012. [Cited 2020 Sep 25]. Available from URL: <http://www.cdc.gov/HAI/settings/lab/labesbl.html>
4. Bradford PA. Extended-spectrum-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clinical microbiology reviews*. 2001;14(4):933-951.
5. Ali AM, Abbasi SA, Ahmed M. Frequency Of Extended Spectrum Beta-Lactamases (Esbl) Producing Nosocomial Isolates In A Tertiary Care Hospital In Rawalpindi. *Pakistan Armed Forces Medical Journal*. 2009; 59(2):154-158.
6. Bell JM, Turnidge JD, Gales AC, Pfaller MA, Jones RN, Sentry APAC Study Group. Prevalence of extended spectrum beta-lactamase (ESBL)-producing clinical isolates in the Asia-Pacific region and South Africa: regional results from SENTRY Antimicrobial Surveillance Program (1998-99). *Diagnostic microbiology and infectious disease*. 2002; 42(3):193-198.
7. Hirakata Y, Matsuda J, Miyazaki Y, Kamihira S, Kawakami S, Miyazawa Y, Ono Y, Nakazaki N, Hirata Y, Inoue M, Turnidge JD. Regional variation in the prevalence of extended-spectrum-lactamase-producing clinical isolates in the Asia-Pacific region (SENTRY 1998-2002). *Diagnostic microbiology and infectious disease*. 2005; 52(4):323-329.
8. Farzana R, Shamsuzzaman SM, Mamun KZ, Shears P. Antimicrobial Susceptibility Pattern of Extended Spectrum Beta-Lactamase Producing Gram-Negative Bacteria Isolated from Wound And Urine in a Tertiary Care Hospital, Dhaka City, Bangladesh. *Southeast Asian Journal of Tropical Medicine & Public Health*. 2013; 44(1):96-103.
9. Islam TA, Shamsuzzaman SM, Farzana A. Prevalence and antibiogram of ESBL producing gram negative bacilli isolated from urine in Dhaka Medical College Hospital, Bangladesh. *Bangladesh Journal of Medical Microbiology*. 2015; 9(1):17-21.
10. Chaudhary U, Aggarwal R. Extended spectrum beta-lactamases (ESBL)-An emerging threat to clinical therapeutics. *Indian journal of medical microbiology*. 2004; 22(2):75.
11. Jacoby GA, Sutton LO. Properties of plasmids responsible for production of extended-spectrum beta-lactamases. *Antimicrobial Agents and Chemotherapy*. 1991; 35(1):164-169.
12. Pitout JD. Infections with extended-spectrum-lactamase-producing Enterobacteriaceae. *Drugs*. 2010; 70(3):313-333.
13. Begum S, Salam MA, Alam KF, Begum N, Hassan P, Haq JA. Detection of extended spectrum beta-lactamase in *Pseudomonas* spp. isolated from two tertiary care hospitals in Bangladesh. *BMC research notes*. 2013; 6(1):1-4.
14. Baron EJ, Peterson LR, Tenover FC, Tenover MC, Tenover JC, Tenover LC, Peterson LR, Finne SM. Enterobacteriaceae. In: Forbes BA, Sahm DF, Weissfeld AS (eds). *Bailey and Scott's diagnostic microbiology*. 9th ed. St Louis: Mosby. 1994; 374-379.
15. Clinical and Laboratory Standard Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fifth Informational Supplement. CLSI document M100-S25. Wayne, PA: CLSI; 2015.
16. Brink AJ, Bizo D, Boffard KD, Feldman C, Grolman DC, Pretorius J, Richards GA, Steyn E. Guideline summary: Appropriate use of tigecycline. *South African Journal of Surgery*. 2012; 50(1):20-21.
17. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum-lactamases conferring transferable resistance to newer-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. *Clinical Infectious Diseases*. 1988; 10(4):867-878.
18. Rahman A. Detection of aerobic bacteria and *Candida* in neonatal sepsis blood with their antibiotic susceptibility pattern and distribution of drug resistance genes among the isolated bacteria by PCR in NICU of Dhaka Medical College Hospital. [M. Phil thesis]. DMC: Bangabandhu Sheikh Mujib Medical University; 2015.
19. Chelliah A, Thyagarajan R, Katragadda R, Leela KV, Babu RN. Isolation of MRSA, ESBL and AmpC-lactamases from neonatal sepsis at a tertiary care hospital. *Journal of Clinical and Diagnostic Research*. 2014; 8(6): 25-27.

20. Cantón R, Novais A, Valverde A, Machado E, Peixe L, Baquero F, Coque TM. Prevalence and spread of extended-spectrum lactamase producing Enterobacteriaceae in Europe. *Clinical Microbiology and Infection*. 2008;14:144-153.
21. Islam S. Detection of extended-spectrum beta-lactamases producing organisms with their phenotypic confirmation by E test and susceptibility to quinolone and fluoroquinolones. Dhaka. [Doctoral dissertation]. The University of Dhaka; 2008.
22. Biswas SM, Mia MR, Ara N, Ibrahim M, Nasir TA, Yunus S. Comparison of Three Dimensional Test and Double Disc Synergy Test for detection of Extended Spectrum beta-Lactamase (ESBL) producing Gram negative bacteria. *Pulse*. 2013;6(1-2):12-19.
23. Shamsuzzaman SM. Emergence of CTX-M-15 producing *E. coli* O25b-ST131 clone in a tertiary care hospital of Bangladesh. *The Malaysian journal of pathology*. 2016; 38(3):241.
24. Kateregga JN, Kantume R, Atuhaire C, Lubowa MN, Ndukui JG. Phenotypic expression and prevalence of ESBL-producing Enterobacteriaceae in samples collected from patients in various wards of Mulago Hospital, Uganda. *BMC pharmacology and Toxicology*. 2015; 16(1):14.
25. Jobayer M, Afroz Z, Nahar SS, Begum A, Begum SA, Shamsuzzaman SM. Antimicrobial susceptibility pattern of extended-spectrum beta-lactamases producing organisms isolated in a tertiary care hospital, Bangladesh. *International Journal of Applied and Basic Medical Research*. 2017; 7(3):189.
26. Fernández-Canigia L, Dowzicky MJ. Susceptibility of important Gram-negative pathogens to tigecycline and other antibiotics in Latin America between 2004 and 2010. *Annals of clinical microbiology and antimicrobials*. 2012; 11(1):29.
27. Kumar D, Singh AK, Ali MR, Chander Y. Antimicrobial susceptibility profile of extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* from various clinical samples. *Infectious Diseases: Research and Treatment*. 2014;
28. Yao F, Qian Y, Chen S, Wang P, Huang Y. Incidence of extended-spectrum beta-lactamases and characterization of integrons in extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolated in Shantou, China. *Acta Biochimica et Biophysica Sinica*. 2007; 39(7):527-532.
29. Adler M. Mechanisms and Dynamics of Carbapenem Resistance in *Escherichia coli*. [Dissertation]. Uppsala University; 2014.