Chattagram Maa-O-Shishu Hospital Medical College Journal
Volume 23, Issue 2, July 2024

Original Article

Detection of Extended Spectrum Beta Lactamase Producing Gram Negative Bacteria in a Tertiary Care Hospital, Chattogram

Sharmin Akhtar[1*]
Md. Ehsanul Haque[1]
Abul Kalam[1]
Md. Musfequr Rahman[2]
Boosra Harun Prima[3]

[1]Department of Microbiology
Chittagong Medical College
Chattogram, Bangladesh.

[2]Department of Aneasthesiology
Cox's Bazar Medical College
Cox's Bazar, Bangladesh.

[3]Department of Microbiology
Chattagram Maa-O-Shishu Hospital Medical College
Chattogram, Bangladesh.

Abstract

Background: *The frequency of Extended Spectrum Beta-Lactamase (ESBL) producing bacteria among clinical isolates is a serious global health concern that has complicated treatment strategies and is very alarming. So the reporting of ESBLproducing Gram negative bacteria from the clinical samples will be useful for the clinician to select the appropriate antibiotics and to take proper precaution for prevention of these resistant organisms. To study the prevalence of ESBL producers among the Gram negative bacteria and antibiotic susceptibility pattern of these isolates.*

Materials and methods: *This cross sectional study was carried out in the Department of Microbiology of a tertiary care hospital, Chattogram from January 2021 to December 2021. A total of ninety consecutive, nonrepetitive, Gram negative isolates were selected as confirmed ESBL producers, detected by Phenotypic Confirmatory Disc Diffusion Test (PCDDT). Antibiotic susceptibility test was performed on Mueller Hinton agar plate by Kirby Bauer Disc Diffusion method.*

Results: *Out of 300 isolates, 90 (52.63%) were found to be ESBL producers by PCDDT. The isolates of Pseudomonas spp. (37.77%) were the most common ESBL producing bacteria followed by Klebsiella spp. (28.88%), Escherichia coli 39 (22.22%) and others. Maximum (47.93%) ESBL producing bacteria were isolated from wound-swab followed by urine (26.44%). Most ESBL producers were resistant to commonly used antibiotics. Amikacin (80%), pipercillin-tazobactam(75%) and meropenem (73%) were the most effective agents for the treatment of ESBL producing bacteria.*

Conclusion: *The findings of this study emphasize the need for a continuous surveillance such as detection of ESBL along with routine susceptibility test will help the clinician to give a strict guideline for antibiotic therapy and reduce the increasing burden of antibiotic resistance.*

Key words: Antimicrobial resistance; ESBL; PCDDT; MDR.

INTRODUCTION

"Antibiotic golden age" is ending in 20th century but the rate of Antimicrobial Resistance (AMR) are rising globally.It is considered as one of the most pressing issues in the global health-care sector due to greater access to antibiotic drugs in developing countries.[1] Gram negative bacteria have developed the broadest spectrum of resistance due to multiple structural adaptions and antibiotic degradation enzymes including ESBL, AmpC Cephalosporinase and Carbapenemase. ESBLs are enzymes that mediate resistance to broad spectrum of beta-lactam antibiotics such as penicillins, third generation of cephalosporins (e.g. Ceftazidime, cefotaxime and ceftriaxone) and aztreonam, but not to cephamycin (Cefoxitin and cefotetan) and carbapenem but are being inhibited by beta lactamase inhibitors like clavulanic acid.[2]

*Correspondence to:
Dr. Sharmin Akhtar
Lecturer
Department of Microbiology
Chittagong Medical College
Chattogram, Bangladesh.
Mobile : +88 01711 30 81 31
Email : Sharminakhtar100@gmail.com

Date of Submission : 10.06.2024
Date of Acceptance : 13.07.2024

www.banglajol.info/index.php/CMOSHMCJ

Infections caused by bacteria carrying ESBL with other resistant determinants have been associated with increased rates of mortality, hospital stay, therapeutic failure, and health costs.[3,4]

During the past decade, ESBL producing Gram-negative bacilli especially Escherichia coli and Klebsiella pneumoniae have emerged as serious pathogens both in hospital and community acquired infections worldwide. Recently ESBL producing Proteus spp., Pseudomonas spp. and Acinetobacter spp. have been reported in the most parts of the world. The occurrence of ESBL among clinical isolates vary greatly world wide and geographically and are rapidly changing over time.[5]

Detection and identification of ESBL producing bacteria and the knowledge of their resistance are of paramount importance in selecting appropriate antimicrobials to be used in the treatment of infection caused by Multi Drug Resistance (MDR) bacteria. Diagnostic laboratories are in need of reliable, cost effective and less labor intensive method for the detection of ESBL-producing bacteria. So this study was performed to investigate the prevalence of ESBL producers from wound-swab and urine samples and also find out the antibiotic resistance patterns among Gram negative bacteria.

MATERIALS AND METHODS

This cross sectional study was carried out in the Department of Microbiology of a tertiary care hospital, Chattogram from January 2021 to December 2021 after getting approval from Research Review Committee (RRC) and Ethical Review Committee (ERC) of Chittagong Medical College. Written consent was taken from all participants. A total of 300 clinical samples were collected from patients admitted in department of medicine, surgery, gynae and obstetrics, burn and plastic surgery unit.

Isolation of gram-negative bacteria

All the wound swabs and urine samples were inoculated on sheep blood agar and MacConkey agar and incubated at 37ºC aerobically for 24 hours. Incubated plates were then examined for the presence of bacterial growth. Organisms were identified by colony morphology, hemolytic criteria, staining characteristics, pigment production and biochemical tests.[6]

Antimicrobial susceptibility testing and screening of ESBL producers

According to CLSI guidelines, the antimicrobial susceptibility pattern was determined by disk-diffusion technique using commercially available antibiotic disks (Oxoid, Hampshire, UK).[7] Escherichia coli ATCC 25922 was used for quality control. ESBL producers were screened by disk-diffusion method using ceftazidime and ceftriaxone. If the isolates are resistant to both of these drugs, they are considered as suspected ESBL producers.[8]

Phenotypic Confirmatory Disc Diffusion Test (PCDDT) for detection of ESBL (CLSI, 2021):

The organisms which had the ability to hydrolyze the third generation of cephalosporins (Ceftazidime and ceftriaxone) were subjected on this method. Mueller- Hinton agar plate was inoculated with standard inoculums (0.5 Mc.Farland) of the test isolate. Disks containing both ceftazidime (30µg) and cefotaxime (30µg) alone and in combination with clavulanate (30/10µg) were placed on the Mueller-Hinton Agar (MHA) plate at a distance (Edge to edge) of 20mm. After placing the discs, they were incubated at 35 C±2 C ambient air for 16-18 hours. A ≥5mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent when tested alone was confirmed ESBL production. When performing the ESBL test,E.coli ATCC 25922 will be used for routine Quality Control.

RESULTS

Out of 300 samples (150 urine and 150 wound-swab), 171 (57%) Gram negative bacteria were isolated. Among Gram negative isolates, *Pseudomonas spp.* was the predominant bacteria 65(38.01%) followed by *Klebsiella spp.* 46 (26.90), *Escherichia coli* 39 (22.80%) *Acinetobacter spp.* 19 (11.11%) and *Proteus spp.* 2 (1.69%). Antimicrobial susceptibility test showed the highest resistance towards ampicillin 98.20%, ciprofloxacin 78.36%, ceftriaxone 71.34% and ceftazidime 70.76% whereas least resistance towards amikacin 18.71%, pipercillin-tazobactam 25.73% and meropenem 27.48%, also nitrofurantoin showed 13.45% resistance against uropathogens. Among 70.76% third generation of cephalosporin resistant Gram negative bacteria, 52.63% isolates were ESBL producersas detected by phenotypic confirmatory disk diffusion test. The highest percentage of ESBL producing bacteria, was *Pseudomonas spp.* 37.77%, followed by *Klebsiella spp.* 28.88%, *Escherichia coli* 22.22%, *Acinetobacter spp.* 10.00% and *Proteus spp.* 1.11%.

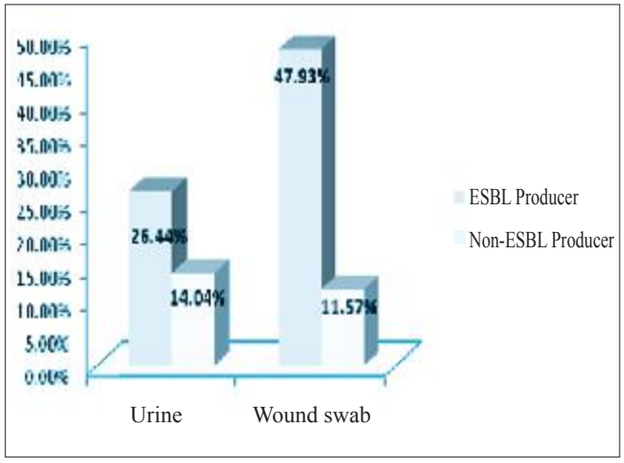

Figure 1 Comparison of ESBL producers between bacterial isolates from urine and wound-swab

Table 1 Antibiotic resistance pattern of isolated Gram negative bacteria (n=171)

Antibiotic disks	Escherichia coli (n = 39)	Klebsiell aspp. (n = 46)	Pseudomonas spp. (n = 65)	Acinetobacter spp. (n = 19)	Proteus spp. (n = 2)
Ampicillin	39 (100.0%)	44 (95.6%)	64 (98.5%)	19 (100.0%)	2 (100.0%)
Ciprofloxacin	35 (89.74%)	35 (76.1%)	46 (70.76%)	16 (84.2%)	2 (100.0%)
Cotrimoxazole	22 (56.4%)	30 (65.2%)	43 (66.1%)	15 (78.9%)	2 (100.0%)
Nitrofurantoin	8 (28.57%)	5 (25%)	7 (43.75%)	3 (75%)	0 (0%)
Gentamycin	24 (61.5%)	29 (63.0%)	39 (60.0%)	13 (68.4%)	2 (100.0%)
Amikacin	8 (20.5%)	9 (19.6%)	6 (9.2%)	8 (42.1%)	1 (50.0%)
Ceftriaxonene	28 (71.8%)	34 (73.9%)	44 (67.7%)	14 (73.7%)	2 (100.0%)
Ceftazidime	28 (71.8%)	34 (73.9%)	43 (66.1%)	14 (73.7%)	2 (100.0%)
Cefuroxime	30 (76.9%)	36 (78.3%)	51 (78.5%)	14 (73.7%)	2 (100.0%)
Cefipime	15 (38.5%)	18 (39.1%)	25 (38.5%)	8 (42.1%)	1 (50.0%)
Aztreonam	30 (76.9%)	36 (78.3%)	44 (67.7%)	15 (78.9%)	2 (100.0%)
Meropenem	11 (28.2%)	13 (28.3%)	17 (26.1%)	6 (31.6%)	0 (0%)
Amoxyclav	24 (61.5%)	30 (65.2%)	39 (60.0%)	13 (68.4%)	2 (100.0%)
Pipercillin-Tazobactam	13 (33.3%)	11 (23.9%)	11 (16.92%)	8 (42.1%)	1 (50.0%)

*Figures within parentheses indicate percentage of resistance.

Table II Distribution of ESBL producers among the isolates (n=90)

Name of Organism	ESBL detected byPCDDT Urine(n=32)	Wound swab(n=58)	Total(n=90)
Escherichia coli	14 (43.75%)	6(10.34%)	20 (22.22%)
Klebsiella spp.	10(31.25%)	16(27.58%)	26 (28.88%)
Pseudomonas spp.	7 (21.87%)	27 (46.55%)	34 (37.77%)
Acinetobacter spp.	1(3.12%)	8(13.79%)	9 (10.00%)
Proteus spp.	-	1(1.72%)	1(1.11%)
Total	32(100%)	58(100%)	90(100%)

Maximum (47.93%) ESBL producing bacteria were isolated from wound-swab followed by urine (26.44%). Highest ESBL producers (52.53%) were isolated from admitted patients of different surgery units specially burn and plastic surgery unit.

DISCUSSION

The spread of ESBL producing bacteria has become strikingly worldwide, indicating that continuous monitoring systems and effective infection control measures are absolutely required. Presence of ESBL compromise the activity of wide-spectrum antibiotics creating major therapeutic difficulties with a significant impact on the outcome of patients.[9]

The prevalence of ESBL producers vary from country to country and it is more common in South-East Asia.[10] In Bangladesh, rate of ESBL producing bacteria isolated were 25% in 2013 and 51% in 2020.[11,12] In our present study, ESBL producing Gram negative bacteria were 52.63%. Similar findings were found in India by Sexena et al. who found 48% and in Nepal, Shilpakar et al. noted 51.10% of the gram negative bacteria were ESBL producers.[13] In current study, the number of ESBL is less than that previously reported by Dalela et al.(2012)in India who found 61.6% ESBL producing Gram negative bacteria and this variation could be due to the geographical changes.[14] In this study, we found maximum number of ESBL producing organisms which were derived from indoor patients. This could be due to the fact that before the arrival in hospital unnecessary prescription by physicians, infections caused by nosocomial organisms in admitted patients, the continuous exposure to the hospital environment or have prosthetic devices makes them more susceptible to infection.

In Bangladesh, the prevalence of ESBL producer among different organisms varied in different studies and it may be due to infection causing bacteria vary from area to area and even hospital to hospital. The high resistance profile of the isolates in this study was a reflection of the high incidence of ESBL isolates that observed in *Pseudomonas spp.* 37.7% which correlated well with other studies as 37.3% and 42.3%.[15,16]

This tertiary care hospital deals with a large number of patients including surgical departments and also burn unit. So, huge number of wound swab may yielded the growth of maximum ESBL producers. Many of these patients were receiving long-time treatment and frequent antibiotic switch without culture sensitivity. Organisms may develop resistance during prolonged antimicrobial therapy and initially susceptible bacteria may become resistant within few days after initiation of treatment.

So in our study, we found ampicillin was ineffective that showed the highest resistance (100%). Susceptibility finding of isolates against cephalosporins and quinolones reported a substantial increase in their resistance. However nitrofurantoin was effective against uropathogens and this can be considered as the first line therapeutic regimen for UTI in our setting. Meropenem would be useful as secondary therapy for MDR and complicated UTI. On the other hand. amikacin has good activity against clinically important gram negative bacteria and the pattern is consistent with other studies in Bangladesh.[17,18] The reason behind such low resistance might be the less use of this antibiotic in this hospital. In the present study, 82% isolates were susceptible to amikacin followed by 75% pipercillin-tazobactum and meropenem.These injectable drugs are not usually used outside of hospital settings, and they are considered mainly as reserved drugs and are being used for those who are resistant to most other antibiotics. Contrary the occurrence of the most common drug resistance due to ESBL is located on a plasmid that can be transferred from one organism to another easily and can incorporate genetic material coding for resistance to other antimicrobial classes.

Early detection and appropriate antibiotic application remain a significant priority in controlling the development and spread of ESBL producing organisms. The phenotypic confirmatory disk diffusion test is more sensitive and better than double disk synergy test in the detection of ESBLs. So Clinical laboratory and standards institute recommended phenotypic confirmatory disc diffusion test for the detection of ESBL

CONCLUSION

The current emergence of ESBL-producing Gram negative bacteria is of concern because its containment is more challenging in developing countries due to poor antimicrobial resistance surveillance and irrational use of antibiotics. So effective measures such as the establishment of active surveillance and infection control programmes, emphasizing hand hygiene together with coherent antibiotic policies in hospitals and clinics should be implemented to stop and manage the spread ESBL producing bacteria in hospitals.

ACKNOWLEDGEMENTS

Department of Microbiology, Chittagong Medical College, Chattogram provided laboratory support to perform this study.

DISCLOSURE

All the authors declared no competing interest.

REFERENCES

1. Byarugaba DK. A view on antimicrobial resistance in developing countries and responsible risk factors. Int J Antimicrob Agents. 2004;24:105–110.
2. Al-Muharrmi, Z., Rafay, A.,Balkhair, A.and Jabri, A. Antibiotic combination as empirical therapy for extended spectrum beta-lactamase.Oman Med J. 2008;23(2):78-81.
3. E. M. Trecarichi, R. Cauda, and M. Tumbarello, "Detecting risk and predicting patient mortality in patients with extended-spectrum β-lactamase-producing Enterobacteriaceae blood stream infections,"Future Microbiology. 2012;7(10):1173-1189.
4. D. L. Maraskolhe, V. S. Deotale, D. K. Mendiratta, and P. Narang, Comparision of three laboratory tests for detection of Ampc β lactamases in Klebsiellaspecies and *Escherichia coli*,"Journal of Clinical and Diagnostic Research. v2014;8:5-8.
5. Babypadmini S, Appalaraju B. Extended spectrum lactamase in urinary isolates of Escherichia coliand Klebsiella pneumoniae – prevalence and susceptibility pattern in a Tertiary care hospital. Indian J. Med. Microbiol. 2004; 22: 172-174.
6. Cheesbrough, M. Microbiological test. In: M. (editor). District Laboratory Practise in Tropical Countries, part 2, 2nd ed. Cambridge University press, India. 2009;35-138.
7. CLSI (2021). Performance Standards for Antimicrobial Susceptibility Testing CLSI Supplement M100, 31th Edn. Wayne, PA: Clinical and Laboratory Standards Institute. 2021.
8. EUCAST (2017) EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance, Version 2.01. 2017;13.
9. Saxena, A., Singh,S., Goyal, R. and Saxena, S. Antibiotic susceptibility pattern of extended spectrum -lactamase ESBL producing gram negative bacilli in a tertiary care teaching hospital, Bareilly, India. Int J Basic Clin Pharmacol. 2019;8(2):228-233.
10. Canton, R., Akova, M., Carmeli, Y., Giske, C.G., Glupczynski, Y. and Gniadkowks, M. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. Clin Microbiol Infect. 2012;18: 413–431.
11. Farzana, R., Shamsuzzaman, S.M., Mamun, K.Z. and 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. J Trop Med Public Health. 2013;44(1):96-103.
12. Mazumder, R. High Prevalence of $bla_{CTX-M-15}$ gene among Extended-Spectrum β -Lactamase-producing Escherichia coli isolates causing extraintestinal infections in Bangladesh. Antibiotics. 2020;9(11):796.
13. Shilpakar, A., Ansari, M., Rai, K.R., Rai, G. and Rai, S.K. Prevalence ofmultidrug-resistant and extended-spectrum beta-lactamase producing Gram-negative isolates from clinical samples in a tertiary care hospital of Nepal Trop Med Health. 2021;49: 23.
14. Dalela, G. Prevalence of Extended Spectrum Beta Lactamase (ESBL) producers among Gram negative bacilli from various clinical isolates in a tertiary care hospital at Jhalawar, Rajasthan, India. J of Clin.& Diagnostic Research. 2012;6(2):182-187.
15. V. Sudha Rani, R. Kondal Rao, S. Ravinder, P. Kanakadurga. Prevalence of Extended Spectrum Beta Lactamase (ESBL) producing Pseudomonas aeruginosa isolates from burns patients. Int. Journal of Contemporary Medical Research. 2016;3(5):1297-1300.
16. Goel,V., Hogade, S.A., Karadesai, S.G. Prevalence of extended spectrum beta lactamase, AmpC betalactamase and metallobetalactamases producing Pseudomonas aeruginosa and Acinetobacterbaumannii in intensive care unit in a tertiary care unit in tertiary care hospital. Journal of Scientific Society. 2013;40(1):28-31.
17. Jobayer, M., Afroz, Z., Nahar, S.S., Begum, A., Begum, S.A., Shamsuzzaman, S.M. Antimicrobial susceptibility pattern of Extended Spectrum Beta-Lactamases producing organisms isolated in a tertiary care hospital, Bangladesh. Int J Applied Basic Med Res. 2017;7(3):189-192.
18. Sarker JN, Bakar SM, Barua R, Sultana H, Anwar S, Saleh AA, et al. Susceptibility pattern of extended spectrum -Lactamase (ESBL) producing Escherichia coli, Klebsiella spp. and Enterobacter spp. to Ciprofloxacin, amikacin and imipenem. J Sci Res Rep. 2015;8:1–9.