VOLUME - 11, ISSUE - 11, NOVEMBER - 2022 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Medical Microbiology

ESBL,MBL AND AMP-C β LACTAMASES PRODUCING SUPERBUGS - AN EMERGING THREAT IN INTENSIVE CARE UNITS OF A TERTIARY CARE HOSPITAL

Harini Sakthi S MBBS Student,Institute of Microbiology, Madurai Medical College, Madurai - 625020, Tamil Nadu, India

Dr. Rammurugan N M.D., Associate Professor, Institute of Microbiology, Madurai Medical College, Tirunelveli - 627011, Tamil Nadu, India

Manjula G M.D., Assistant Professor, Institute of Microbiology, Madurai Medical College, Madurai - 625020, Tamil Nadu, India

Sucilathangam G* M.D., Associate Professor, Department of Microbiology, Tirunelveli Medical College, Tirunelveli - 627011, Tamil Nadu, India *Corresponding Author

Mangala Adisesh M.D., Former Director, Institute of Microbiology, Madurai Medical College, Madurai - 625020, Tamil Nadu, India

ABSTRACT

Background: An alarming rise in the rates of the antibiotic resistance has now become a serious and an increasingly common public health concern, with severe implications, especially in the Intensive Care Units. A variety of β-lactamases which include ESBLs, Amp C β-lactamases and metallo-βlactamases, have emerged as the most worrisome mechanism of resistance among the gram negative bacteria, which pose a therapeutic challenge to the health care settings. **Materials and methods:** The present study was aimed at knowing the prevalence of various β-lactamases in the gram negative isolates which were obtained from ICU patients. A total of 90 clinical samples received over a period of 3 months were processed for their identification and their antimicrobial susceptibility pattern was determined. They were then screened for the β-lactamase production. Results: ESBL production was found to be maximum as compared to the other β-lactamases. Of the 90 gram negative isolates, 20(22.22%) were ESBL producers, followed by 6(6.66%) Amp-C producers and 4(4.44%) MBL producers. Coexistence of ESBL and MBL was reported in 6.66% isolates, the Amp-C and the MBL co production was shown by 4.44% isolates whereas the Amp-C and the ESBL co production was shown in 15.55% isolates. **Conclusion** With the increasing rate of β-lactamase producers in ICUs,Identification of these strains will be useful for physicians to prescribe the most appropriate antibiotic. Continual surveillance of β-lactamase producers that assist appropriate antibiotic therapy and better patient outcomes and also reduces antibiotic resistance through better infection control practices.

KEYWORDS : Intensive Care Units, Gram-negative bacteria , Antibiotic resistance ,Extended Spectrum Beta-Lactamase(ESBL), Metallo-β Lactamases(MBL), Amp C β-lactamases

INTRODUCTION:

The incidence of Nosocomial infections in the Intensive Care Units (ICU) is showing a rising trend, mainly because of the severe clinical conditions which are associated with the impaired immunity, increasing the use of invasive diagnostic procedures, lapses in the sterilization and the disinfection techniques and the indiscriminate use of antibiotics.

The β-lactam antibiotics are among the most frequently prescribed antibiotics in the ICUs world-wide, which are favored because of their efficacy, broad spectra and low toxicity. The selective pressures which are generated by the indiscriminate use of the Beta-lactam antibiotics have led to the selection of a variety of mutated forms of β-lactamases such as the ESBLs, Amp-C β-lactamases and Metallo-β-lactamases which have emerged as the most worrisome resistance mechanism which poses a therapeutic challenge to the health care settings [1].

These "newer β-lactamases" are capable of hydrolyzing a wide range of β-lactams antibiotics, notably the extended-spectrum penicillins and the third and fourth generation cephalosporins, which include the Carbapenams [2]. The ESBL producing organisms also express the Amp-C β-lactamases and they may be co-transferred with the plasmids, thus mediating the Fluoroquinolone and the amino glycoside resistance. The treatment options are fast running out, particularly against the gram negative nosocomial pathogens [3]. They are of significant concern because they restrict the therapeutic options, cause treatment failures and are increasing in occurrence worldwide. These enzymes are associated with the potentially fatal laboratory reports of a false susceptibility to the Cephalosporins, that can lead to the prescription of the inappropriate therapy for the infected patients.[4]

Though a number of phenotypic methods have been proposed, the coexistence of different classes of □-lactamases in a single bacterial isolate may pose diagnostic and treatment challenges. Hence, it is necessary to know the accurate prevalence of the β-lactamase producing strains in the high risk areas, so as to formulae a policy of the empirical therapy in the ICUs where the infections which are caused by the resistant organisms are much higher [5].

The present study was undertaken to determine the prevalence rates of the ESBL, AmpC and MBL in Gram negative bacteria which produced the β-lactamase enzymes in ICUs, so as to formulate an antimicrobial policy on the basis of the local epidemiological data.

Objectives:

1. To determine the prevalence of ESBL, Amp-C and MBL in Gram-negative bacteria isolated from clinical specimens of ICU patients.
2. To evaluate various phenotypic methods for identification of ESBL, Amp-C and MBL production.
3. To formulate an antimicrobial policy on the basis of the local epidemiological data to aid in prompt and precise patient management.

Methodology:

This cross sectional study was conducted at Department of Microbiology, Intensive Care Units of Medicine, Surgery of Madurai Medical College Hospital, Madurai, Tamil Nadu for the period of three months from June 2021 to August 2021.

Institutional ethical committee clearance was obtained before the commencement of the study. All patients >18 years of age consecutively admitted to Intensive Medical care unit. Clinical information including age, sex, occupation, previous hospitalization, history of antibiotic use, current hospital stay, type of sample, invasive procedures undergone (if any) were recorded for each patient. Samples from GRH were received in the clinical bacteriology laboratory and were processed for microbiological confirmation of clinically suspected infection. Patients in whom Gram negative bacteria were isolated during routine diagnostic testing were included in the study.

Sample Collection and processing

Samples were collected from patients as per standard procedures. All the isolates were identified by the standard microbiological tests. The antimicrobial susceptibility testing of the isolates were determined by the Kirby Bauer disc diffusion method according to the CLSI guidelines [6]. The reference strains, ESBL positive *Klebsiella pneumoniae* ATCC 700603 and ESBL negative *Escherichia coli* ATCC 25922 were included in the study.**(Figure-1)**

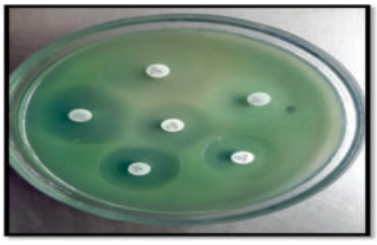

Figure-1Antibiotic susceptibility testing by Kirby Bauer's Disc Diffusion method:

Detection of the ESBLs

Each strain was screened for the ESBL production against Cefotaxime, Ceftazidime and Cefpodoxime. The strains which were resistant to these third generation Cephalosporins were confirmed by three phenotypic tests i.e. the disc potentiation test (by using Ceftazidime and Ceftazidime-clav discs), the double disc synergy test .**(Figure-2)** and MIC by E-test as per the CLSI guidelines [6]. .**(Figure-3)**

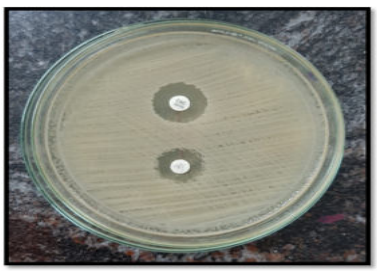

Figure-2 phenotypic confirmatory combination disc diffusion test

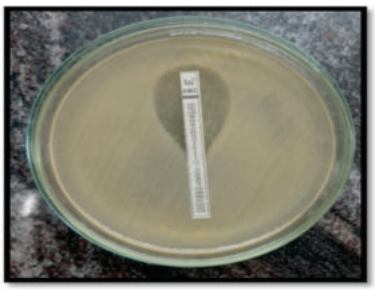

Figure-3 MIC by E-test

Detection of the Amp-C β-lactamases:

All the strains were screened for the Amp-C β-lactamase production by the disc antagonism test. The isolates which showed a reduced susceptibility to Cefoxitin were tested for confirmation by the modified three dimensional test. An indentation or a flattening of the zone of inhibition indicated the Amp-C production.[7].**(Figure-4)**

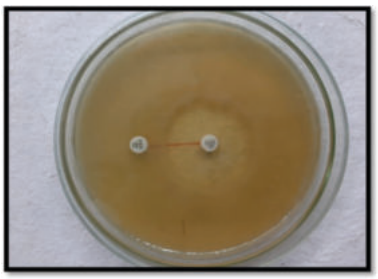

Figure 4 : Amp-C Beta lactamases screening assay using 30µg Cefoxitin disc

Detection of the Metallo- β- lactamases (MBLs)

The Metallo- β- lactamase production was detected by the Ceftazidime – EDTA and the Imipenem – EDTA double disc synergy test. The organisms were considered to be MBL producers if the increase in the inhibition zone of the β-lactam+EDTA disk was ≥ 5 mm.[8] **(Figure-5)**

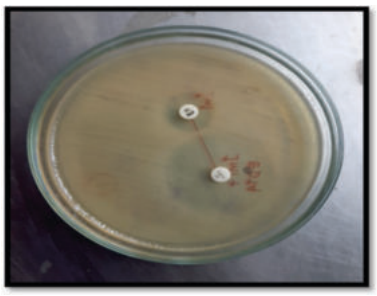

Figure 5 : Imipenem – EDTA double disc synergy test

Statistical Analysis

All the results obtained were analyzed statistically for their completeness, consistency and accuracy by the parameters like mean and percentages.

The correlation of Interleukin-6 level with blood culture for neonatal sepsis was compared statistically and results were analyzed by IBM SPSS Statistics 20. Chi-square test and Fisher Exact test were used in calculating the P-value. The P-Values of less than 0.05 were considered as statistically significant (P<0.05).

RESULTS:

The study was conducted in Department of Microbiology and Medicine and Surgery (Intensive Medical care unit)at Government Rajaji hospital, Madurai Medical College from June 2021 and August 2021(3 months).

Ninety patients were included in the study. Urine and Pus samples were cultured. Out of these 90 cases, 64(71.11%) cases were Males and 26(28.89%) cases were Females. Most of cases was in the age group of <60 years(62.22%) and followed by above 60 years(37.78%).

β lactamase production in ICU:

The skin and soft tissue infections (70%) were the most common infections, followed by urinary tract infections (65%) in the ICUs.

Among the 90 gram negative isolates, the β lactamase production was detected in 66 (73.33%) isolates and the prevalence of the Beta lactamases in the respective ICUs was determined. It was found to be maximum in the surgical ICU (69.7%) followed by Medical ICU (30.3 %).**(Figure-6)**

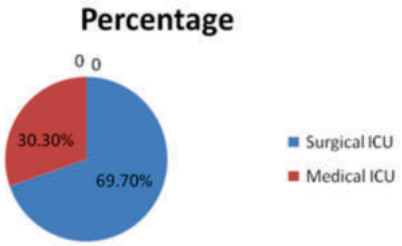

Figure-6 β lactamase production in ICU

Prevalence of ß- lactamase producing isolates in ICUs

Of the 38 strains of *Escherichia coli*, 28 were β lactamases(73.68%) producers,14 were *Pseudomonas aeruginosa* (87.5%) and 12 were *Acinetobacter baumannii* (85.7%). Prevalence of ß- lactamase producing isolates in ICUs was depicted in **Table -1.**

Table-1 Prevalence of ß- lactamase producing isolates in ICUs

Organism	Total no. of GNB	Resistant Strains	β lactamase producers
E coli	38	28	73.68%
Pseudomonas	16	14	87.5%
Klebsiella	14	6	42.85%
Proteus	8	6	75%
Acinetobacter	14	12	85.7%
Total	90	66	73.33%

Frequency of ESBL,AMP-C,MBL Production in Gram negative isolates

Of the 90 gram negative isolates, 20(22.22%) were ESBL producers, followed by 6(6.66%) Amp-C producers and 4(4.44%) MBL producers. The major ESBL producer was *Escherichia coli* (60%), followed by *Pseudomonas aeruginosa* (20%) and *Klebsiella pneumoniae* (10%).The Amp-C production was also maximally seen in *Escherichia coli* (66.67%) and MBL production was also observed in *Escherichia coli* (100%)The co production of the ESBL/MBL/ Amp-C β-lactamases was observed in 12 (13.33%) strains. The ESBL and MBL co production was detected in 6 (6.66%) isolates and it was found to be maximum in *Escherichia coli* (66.67%), *Pseudomonas aeruginosa* (33.33%), while the ESBL and the Amp-C co producers were 14 (15.55%) and they were isolated from *Acinetobacter baumannii* (42.86%).*Escherichia coli* (28.57%), *Klebsiella pneumoniae* (14.28%) and *Proteus mirabilis* (14.28%). The co production of Amp-C and MBL was observed in 4 (4.44%) strains and it was detected in *Pseudomonas aeruginosa* (50%) and *Proteus mirabilis* (50%).**(Table-2/Figure-7)**

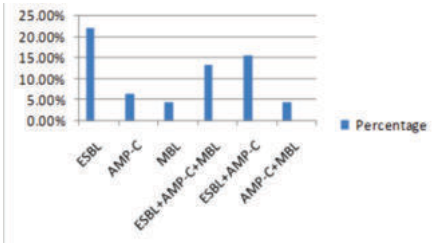

Figure-7 Distribution of ß- lactamases in ICUs

Table-2 Distribution of ß- lactamases in ICUs

Organism	Total	ESBL		AMP-C		MBL		E+A+M		E+M		A+M		E+A	
	isolates	No	%	No	%	No	%	No	%	No	%	No	%	No	%
E coli	38	12	60%	4	66.67%	4	100%	-	-	4	66.67%	-	-	4	28.57%
Pseudomonas	16	4	20%	-	-	-	-	6	50%	2	33.33%	2	50%	-	-
Klebsiella	14	2	10%	-	-	-	-	2	16.67%	-	-	-	-	2	14.29%
Proteus	8	-		-	-	-	-	2	16.67%	-	-	2	50%	2	14.29%
Acinetobacter	14	2	10%	2	33.33%	-	-	2	16.67%	-	-	-	-	6	42.85%
Total	90	20	(22.22%)	6	(6.66%)	4	(4.44%)	12	(13.33%)	6	6.66%	4	4.44%	14	15.55%

A high degree of co-resistance to Ciprofloxacin (72.22%),Gentamicin (66.67%) and Amikacin (44.44%) was observed in the β lactamase producing organisms. All the ESBL/Amp-C/MBL positive isolates were moderately sensitive to Imipenem (64.44%) and the Piperacillin + Tazobactam combination (52.6%).

DISCUSSION:

Infections are among the most important causes of mortality worldwide. Although ICUs constitute only 4–5% beds strength of a hospital, they harbour up to 30% of the Nosocomial infections in the hospital. The organisms or bugs causing ICUs infections are different in low- or middle income countries like India as compared to developed Western world with a variety of ß-lactamases which include ESBLs, Amp-C ß-lactamases and Metallo-ßlactamases.They are associated with a significant morbidity and mortality.[9]

Inadequate infection control facilities in the hospitals, due to lack of resources, ignorance about the gravity of the situation or the obvious neglect to follow precautions even in resource rich tertiary care centres, provide a perfect milieu and breeding ground for these superbugs. An alarming rise in the rates of the antibiotic resistance has now become a serious and an increasingly common public health concern, with severe implications, especially in the intensive care units which pose a therapeutic challenge to the health care settings.

The numerous lactamases are encoded either by the chromosomal genes or by the transferable genes which are located on the plasmids or the transposones [10]. Initially, these enzymes were commonly found in the *Klebsiella* species and in *E,coli* [11] but now, these enzymes are produced by all members of *Enterobacteriaceae* and other gram negative bacilli.[12]

In the present study, the prevalence of various β-lactamases in the Gram negative bacteria, which included the *Enterobactericeae* and the nonfermenters was 73.33%, which was alarmingly high. The ESBL production was ((22.22%) found to be maximum as compared to the other β-lactamases. The major ESBL producer was *Escherichia coli* (60%), followed by *Pseudomonas aeruginosa* (20%) and *Klebsiella pneumoniae* (10%).Similar findings were reported in a study which was done by Bandekar et al, which showed a high prevalence of the ESBL producers (39.8%) in burn patients [13].

A study which was done by Harakuni et al reported a high prevalence of the ESBLs (74%) in ICU patients.[14]Laghawe et al, in his study, reported 19.67% ESBL producers.[15]It has been proved that the prevalence of the ESBLs among the clinical isolates varies from country to country and institution to institution within the same country.

In the current study, the Amp-C production was seen in 6.66% of *Escherichia coli* isolates as compared to that in other studies that had reported a high prevalence of the Amp-C producers. It was 17.3% in Kolkata [16] and 22.9% in a study which was done by Bandekar et al., [13] in burn patients,

whereas a study which was done by Bhattacharjee et al showed 22% Amp-C producing *Pseudomonas aeruginosa* [17].

The low prevalence of the Amp-C producers in this study could be due to the differences in the geographical distribution, which may have produced variations in the prevalence of the β -lactamases which may have been present in the different organisms, which may have given rise to the varied resistance patterns.

The only β-lactams which were active against the Amp-C and the ESBL co producers were the carbapenems; however, recently, the resistance to the carbapenems has been increasing, which is mostly due to the production of the Metallo β-lactamases. In the present study, the MBL producers were 4.44%. MBL production was observed in *Escherichia coli* (100%).These findings were in concordance with the study which was done by Bandekar et al, who reported 15.7% MBL producers [13].

The coexistence of different classes of β-lactamases in a single bacterial isolate may pose diagnostic and treatment challenges. The Amp-C producing organisms can act as a hidden reservoir for the ESBLs. Also, the high-level expression of the Amp-C -lactamases may mask the recognition of the ESBLs and it may result in a fatal and an inappropriate antimicrobial therapy.

In the present study, coexistence of ESBL and MBL was reported in 6.66% isolates, the Amp-C and the MBL co production was shown by 4.44% isolates whereas the Amp-C and the ESBL co production was shown in 15.55% isolates. This was in contrary to a study which was done by Arora et al reported the Amp-C and MBL coproduction in 46.6% isolates and the ESBL and Amp-C co production in 3.3% isolates [15].

The increase in the prevalence of the Amp-C, MBL and the ESBL producing isolates may be indicative of the ominous trend of more and more isolates acquiring the resistance mechanisms, thus rendering the antimicrobial armamarium ineffective. In this study, the multidrug resistant strains showed co resistance to the Fluoroquinolones and the Amino glycosides, but they were moderately susceptible to Imipenem and the Piperacillin+Tazobactem combination, which was in concordance with the findings of other studies [18-20].

CONCLUSION:

This study revealed high rates of β-lactamase producers in patients admitted to the ICU of a tertiary hospital. It is useful to develop its own antimicrobial stewardship program which should be based on the local epidemiological data and international guidelines, to optimize the antimicrobial use among the hospitalized patients. It will be helpful to improve the patient outcomes, to ensure a cost effective therapy, and to reduce the adverse consequences of the antimicrobial use. The high prevalence of these organisms in the ICUs emphasizes the need for an early detection of the β-lactamase producing organisms by simple screening methods, which can help in providing an appropriate antimicrobial therapy and in avoiding the development and the dissemination of these multidrug resistant strains. Preventive measures such as a continuous surveillance of the ICUs and a strict implementation of Infection control practices can go a long way in containing the threat of drug resistance in the health-care settings.

Acknowledgement

The financial support for this study was given by Indian Council of Medical Research (ICMR), New Delhi for Short Term Studentship(STS)- 2020.We are also thankful to the Dean, staff members of Microbiology and Intensive Care Units of Medicine, Surgery at Madurai Medical College for their contribution during laboratory investigation and data collection.

REFERENCES:

1. Deshmukh DG, Damle AS, Bajaj JK, Bhakre JB. The metallo β lactamase producing clinical isolates from the patients of a tertiary care hospital. Journal of Laboratory Physicians. 2011;3(2):93–97.
2. Khan MKR, Thukral SS, Gaind R. Evaluation of the modified double-isc synergy test for the detection of ESBLs in the Amp-C β lactamase- producing Proteus mirabilis. Ind J Med Microbial. 2008;26(1):58–61.
3. Livermore DM. Has the era of untreatable infections arrived? J Antimicrob Chemother. 2009;64:29–36.
4. Khan MKR, Thukral SS, Gaind R. Evaluation of the modified doubleisc synergy test for the detection of ESBLs in the Amp-C β lactamase- producing Proteus mirabilis. Ind J Med Microbial. 2008;26(1):58–61.
5. Pitout JD, Reisbig MD, Venter EC, Church DL, Hanson ND. Modification of the double disc test for the detection of the Enterobacteriaceae which produced the extended spectrum and the Amp-c β lactamases. J Clin microbial. 2003;41:3933–35.
6. CLSI. The performance standards for the antimicrobial disc susceptibility tests. Wayne PA: CLSI; 2005. pp. M100–S15.
7. Shahid S, Malik A, Agrawal M, Singhal S. The phenotypic detection of the extended spectrum and the AmpC β lactamases by a new spot inoculation method and a modified three dimensional extract test. J Antimicrobial Chemother. 2004;54:684–87.
8. Pitout JD, Gregson DB, Poirel L, McClure JA, Le P, Church DL. The detection of Pseudomonas aeruginosa which produced metallo- β lactamases in a large centralized laboratory. J Clin Microbiol. 2005;43:3129–35.
9. Itokazu G, Quinn J, Bell-Dixon C, Kahan F, Weinstein R. The antimicrobial resistance rates among aerobic the gram-negative bacilli which were recovered from the patients in the intensive care units:the evaluation of a national post marketing surveillance program. ClinInfect Dis. 1996; 23:779–84.
10. Mary VJ, Kandathi AJ, Balaji V. Comparison of the methods for the detection of the carbapenamase and the metallo- β lactamases production in the clinical isolates. Ind. J. Med. Res.2005;121:780-83.
11. Mathur P, Kapil A, Das B, Dhawan B. The prevalence of ESBL producing gram negative bacteria in a tertiary care hospital. Ind J Med Res. 2002;115:153-57.
12. Kumar MS, Lakshmi V, Rajagopalan R. The occurrence of extended spectrum β lactamases among the Enterobacteriaceae species which were isolated at a tertiary care institute. Ind J Med Microbiol. 2006;24;208-11.
13. Bandekar N, Vinodkumar CS, Basavarajappa KG, Prabhakar PJ, Nagaraj P. The beta lactamases mediated resistance amongst the gram negative bacilli in burn infections. Int J of Bio Res.2011;2:766-70.
14. Harakuni S, Karadesai SG, Mutnal MB, Metgud SC. The prevalence of the extended spectrum β-lactamase-producing clinical isolates of *Klebsiella pneumoniae* in the intensive care unit patients of a tertiary care hospital. Annals of tropical medicine and health 2011;4:96-98.
15. Laghawe A, Jaitly N, Thombare V. The simultaneous detection of the ESBL and the AmpC b-lactamases in gram negative bacilli. JCDR.2012;6:660-63.
16. Arora S, Bal M. The AmpC β-lactamase producing bacterial isolates at a Kolkata hospital. Ind J Med Res. 2005;122:224-33.
17. Bhattacharjee A, Anupurba S, Guar A, Sen MR. The prevalence of inducible AmpC β- lactamase producing Pseudomonas aeruginosa ina tertiary care hospital in northern India, IJMM 2008;26:89-98.
18. Lautenbach E, Strom BL, Bilker WB, Patel JB, Edelstein PH, Fishman NO. The epidemiological investigation of the fl uroquinilone resistance in the infections which were caused by extended-spectrum betalactamase-producing Escherichia coli and *Klebsiella pneumoniae*. Clin Infect Dis. 2001; 33:1288-94.
19. Subha A, Ananthan S, Alavandi SV. Extended spectrum beta lactamase production and multidrug resistance in the *Klebsiella* species which were isolated from children who were under five years, who had intestinal and extra intestinal infections. Indian J Med Res.2001; 113:181-85.
20. Laxmi V. The need for national/regional guidelines and policies in India to combat antibiotic resistance. Ind J Med M .2008;26(2):105-7.