International Journal of Medical Research Professionals
P-ISSN: 2454-6356; E-ISSN: 2454-6364
DOI: 10.21276/ijmrp

IJMRP

Original Research Article.

Study of Extended Spectrum Beta Lactamase and Metallo Beta Lactamase Production among Gram Negative Clinical Isolates from a Tertiary Care Hospital, North-East India

Binita Bhuyan[1], Pallabi Sargiary[2*], Reema Nath[3]

[1]Demonstrator, [2*]Assistant Professor, [3]Professor and Head,
Department of Microbiology, Jorhat Medical College & Hospital, Jorhat, Assam, India.

ABSTRACT

Introduction: Antibiotic resistance among Gram negative bacteria is a rapidly expanding problem. In Gram-negative bacteria, extended-spectrum beta-lactamases (ESBL), and metallo beta-lactamases (MBL), have emerged as a cause of antimicrobial resistance. ESBL and MBL producing isolates are often multi-drug resistant. The multidrug resistant (MDR) isolates pose threat to therapy as well as serious concerns for infection control management.

Methods: A cross-sectional study was carried out at the Microbiology Laboratory of a tertiary care teaching hospital in Assam, North East India between September 2017 and January 2018. A total of 339 Gram negative isolates were included in the study. Antimicrobial susceptibility testing of the isolates was performed by Kirby-Bauer disc diffusion technique. Phenotypic identification of ESBL producing isolates was carried out using CLSI recommended combined disc diffusion test method. Combined disc diffusion test using Imipenem alone and Imipenem with EDTA discs was done for phenotypic detection of MBL production.

Results: Of the 339 Gram negative isolates, 14.75% (50/339) were ESBL producers, 17.11% (58/339) were MBL producers and 3% (10/339) were both ESBL and MBL producers. *Klebsiella species* was the most commonly isolated ESBL as well as MBL producer. MBL was found in significantly higher percentage of isolates from inpatients than outpatients ($p<0.0001$).

Conclusion: Infections with strains expressing ESBL or MBL are a challenge for both microbiologists and clinicians as they are having less therapeutic options. Early detection of beta-lactamase producing isolates can help in providing an appropriate antimicrobial therapy and in avoiding the development and the dissemination of these multidrug resistant strains.

Key Words: Gram Negative Bacteria, ESBL, MBL, Multidrug Resistant.

***Correspondence to:**

Dr. Pallabi Sargiary,
Department of Microbiology,
Jorhat Medical College & Hospital,
Jorhat, Assam, India.

Article History:

Received: 09-06-2018, **Revised:** 04-07-2018, **Accepted:** 27-07-2018

Access this article online	
Website: www.ijmrp.com	Quick Response code
DOI: 10.21276/ijmrp.2018.4.4.016	

INTRODUCTION

Antibiotic resistance among Gram negative bacteria is a rapidly expanding problem as they are able to mutate, acquire and transmit plasmids and other mobile genetic elements encoding antibiotic resistance gene. During the last two decades, the appearance and widespread dissemination of bacterial infections which are resistant to beta-lactams, especially to 3rd generation cephalosporins and carbapenems, has become a significant problem Worldwide.[1] In Gram-negative bacteria, the newer beta-lactamases, including extended-spectrum beta-lactamases (ESBL), and metallo beta-lactamases (MBL), have emerged as a cause of antimicrobial resistance.[2] ESBL producers are resistant to and can hydrolyze penicillins, cephalosporins, and aztreonam (except for cephamycins or carbapenems) and are inhibited by β-lactamase inhibitors such as clavulanic acid.[3] ESBL producing isolates, often, also exhibit resistance to other classes of drugs such as aminoglycosieds, cotrimoxazole, tetracycline and flouroquinolones.[4] Genes for ESBL production are often located in plasmids and are transferable between strains or different bacterial species.[5] Carbapenem antibiotics have shown remarkable activity against ESBL producing bacteria.[6,7] However, there has been emergence of carbapenem resistant bacteria due to selective pressure by production of carbapenem hydrolysing enzyme, metallo-β-lactamase.[6,8] Metallo-Beta-Lactamase enzymes can hydrolyze a wide variety of beta lactams including penicillins, cephems and carbapenems except aztreonam.[9,10] The multidrug resistant (MDR) isolates pose threat to therapy as well as serious concerns for infection control management.[11] So, the rapid detection of ESBL and MBL-positive gram-negative bacilli is necessary to aid infection control and to prevent their dissemination.[12]

The present study was undertaken to find out the production of Extended spectrum beta lactamase (ESBL) and Metallo beta lactamase (MBL) among the Gram negative isolates from various clinical specimens. Knowledge of antimicrobial susceptibility pattern of these isolates will help in formulating an antimicrobial policy based on the local epidemiological data.

MATERIALS AND METHODS

A cross-sectional study was carried out at the Microbiology Laboratory of a tertiary care teaching hospital in Assam, North East India between September 2017 and January 2018.

A total of 339 consecutive non-repetitive Gram negative bacteria isolated from various clinical specimens received from different wards, OPDs and ICUs were included in the study. Bacterial isolates were identified using standard microbiological techniques.[13]

Antimicrobial susceptibility testing of the isolates was performed by Kirby-Bauer disc diffusion technique and the interpretation was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines (2017).[14] The isolates were tested against the following panel of antibiotics- piperacillin (100 µg), gentamicin (10 µg), amikacin (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), ofloxacin (5 µg), levofloxacin (5 µg), norfloxacin (10 µg), cotrimoxazole (1.25/23.75 µg), amoxicillin/clavulanic acid (20/10 µg), ampicillin/sulbactam (10/10 µg), piperacillin/tazobactam (100/10 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg) and nitrofurantoin (300 µg) (only in urine isolates). All antibiotic discs were obtained from Hi-Media Labs, Mumbai, India. Those isolates showing resistance to one or more extended-spectrum Cephalosporins were further subjected to phenotypic assay for detection of ESBL and MBL production.

Extended Spectrum Beta Lactamase (ESBL) Detection

Phenotypic identification of ESBL producing isolates was carried out using CLSI recommended combined disc diffusion test method.[14] Antibiotic discs containing ceftazidime (30 µg), cefotaxime (30 µg) and in combination with clavulanic acid (ceftazidime + clavulanic acid (30/10 µg) and cefotaxime + clavulanic (30/10 µg) were used. Disks were placed 20 mm apart (centre to centre) on Muller-Hinton agar medium (Hi-Media, Mumbai, India) inoculated with 0.5 McFarland suspension of the tested bacterial isolate. Plates were incubated at 35±2°C for 16-18 hrs. An increase of ⩾5 mm in zone of inhibition around either ceftazidime/clavulanic acid and cefotaxime/clavulanic acid discs than ceftazidime and cefotaxime discs alone was regarded as ESBL positive isolate. *Klebsiella pneumoniae* ATCC 700603 (ESBL producer) and *Escherichia coli* ATCC 25922 (betalactamase negative) were included as control strains in the study.

Metallo-Beta-Lactamase (MBL) Detection

A combined disc test was performed using two imipenem (10 µg) discs, one containing 10 µl of 0.1 M (292 µg) anhydrous EDTA (Sigma Chemicals, St. Louis, MO). Discs were placed 25 mm apart (centre to centre) on Muller-Hinton agar (Hi-Media, Mumbai, India) plates inoculated with 0.5 McFarland suspension of the bacterial isolate tested and plates were incubated for 16-18 hrs at 35°C. An increase in inhibition zone diameter of > 4 mm around the Imienem-EDTA disc compared to that of the imipenem (10 µg) disc alone was considered positive for Metallo- β-lactamase production.[15] A New Delhi Metallo-beta lactamase (NDM) positive (genotypically confirmed) *Klebsiella pneumoniae* clinical isolate and *Pseudomonas aeruginosa* ATCC 27853 were used as positive control and negative control respectively.

Statistical analysis of data was done using Microsoft Excel 2007.

Table 1: Distribution of ESBL and MBL producers

Organism	No. of isolates tested	ESBL producer n (%)	MBL producer n (%)	ESBL+MBL producer n (%)
Klebsiella species	188	30 (16)	40 (21.3)	2 (1.3)
Escherichia coli	81	17 (21)	11 (14.5)	3 (4)
Pseudomonas species	57	2 (3.5)	4 (7)	4 (7)
Proteus species	7	1 (14.3)	0	0
Acinetobacter baumannii	3	0	0	1 (33.3)
Citrobacter koseri	3	0	3 (100)	0
Total	339	50(14.75)	58(17.11)	10 (3)

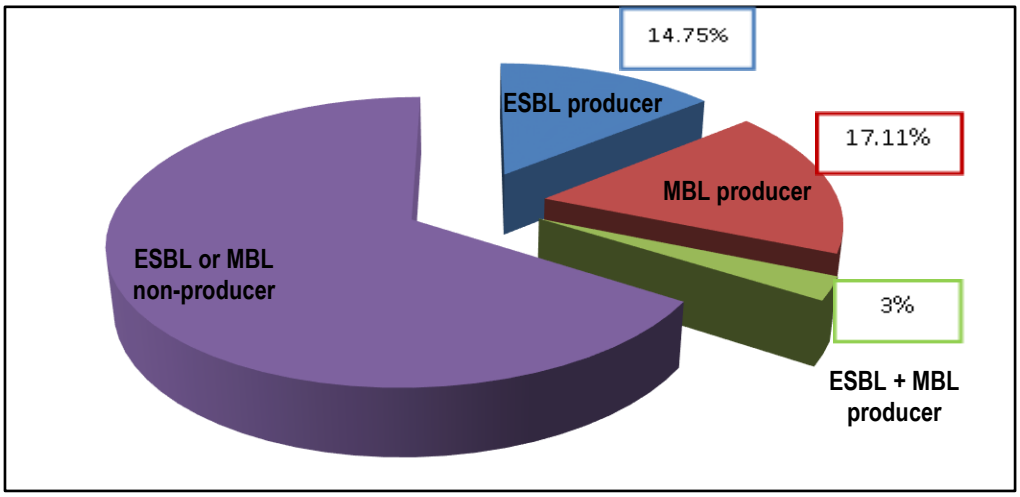

Fig 1: Frequency of ESBL and MBL production amongst the Gram negative isolates (n=339)

Table 2: Sample Wise Distribution of ESBL and MBL producers

Specimen	ESBL producer n (%)	MBL producer n (%)	ESBL+MBL producer n (%)
Urine	27 (54)	30 (51.7)	7 (70)
Sputum	18 (36)	12 (20.7)	2 (22.2)
Pus	4 (8.3)	12 (20.1)	1 (11.1)
***Others**	1 (2.1)	4 (7)	0
Total	50 (100)	58 (100)	10 (100)

*Include throat swab, tracheostomy tube and endotracheal tube specimen.

Table 3: ESBL and MBL production in relation to patients

Patients	No. tested	Positive (%)	Fischer's Exact "p" value
ESBL			
Inpatients	121	16 (13.2)	0.63
Outpatients	218	34 (15.6)	
MBL			
Inpatients	121	44 (36.4)	<0.0001
Outpatients	218	14 (6.4)	
ESBL+MBL			
Inpatients	121	4 (3.3)	0.75
Outpatients	218	6 (2.8%)	

Table 4: Antibiotic resistance pattern of beta lactamase producer organisms

Antibiotic	Resistance (%)
Amoxycillin+Clavulanic acid	98.1
Ceftriaxone	97.5
Piperacillin	95.5
Cefotaxime	93.9
Ceftazidime	93.6
Ampicillin+Salbactam	90.6
Aztreonam	85
Norfloxacin	82.7
Gentamicin	82.6
Cotrimoxazole	81.1
Amikacin	78.8
Ofloxacin	71.9
Piperacillin+Tazobactam	64.9
Levofloxacin	64.3
Meropenem	63.8
Imipenem	42.2
Nitrofurantoin	27.1

RESULTS

A total of 339 Gram negative bacteria isolated from various clinical samples (n=330) between September, 2017 and January, 2018 in the Microbiology Laboratory of a tertiary care teaching hospital in North-East India were included in the study. *Klebsiella spp.* (188/339; 55.5%) was the most common Gram negative bacteria isolated followed by *Escherichia coli* (81/339; 23.9%) and *Pseudomonas spp.* (57/339; 16.8%) [Table 1].

Of the 339 Gram negative isolates, 14.75% (50/339) were ESBL producers, 17.11% (58/339) were MBL producers while 3% (10/339) were both ESBL and MBL producers [Fig 1].

In our study *Klebsiella spp.* was found to be the most commonly isolated ESBL producer, 16% of which produced ESBL. Other commonly isolated ESBL producers were 21% of *Escherichia coli* isolates and 14.3% of *Proteus spp*. Likewise *Klebsiella spp* was also the most commonly isolated MBL producer, 21.3% of which produced the enzyme. Other commonly isolated MBL producers were 14.5% of *Escherichia coli* isolates and 7% of *Pseudomonas spp*. All the three *Citrobacter koseri* isolates were MBL producer (100%). [Table1]. *Psedomonas spp* was the most common bacteria producing both ESBL and MBL.

Majority of the ESBL and MBL producing organisms were isolated from urine (27/50; 54% and 30/58; 51.7% respectively) sample. [Table 2].

As shown in Table 3, among isolates that produced ESBL and/or MBL, only MBL was found in significantly higher percentage of isolates recovered from inpatients than outpatients ($p<0.0001$).

The antibiotic resistance pattern of beta lactamase producing Gram negative isolates is shown in table 4.

It was observed that betalactamase (ESBL and/or MBL) producers were highly resistant to multiple groups of drugs. Carbapenems showed lowest resistance against beta lactamase producers. Nitrofurantoin was the least resistant antibiotic against beta lactamse producers isolated from urine.

DISCUSSION

Increasing prevalence of infections caused by multidrug resistant Gram negative bacilli due to production of ESBL and MBL is a major concern throughout the World. Resistance in gram-negative bacteria is a serious problem and calls for an effective infection control measure to curb their dissemination.[16,17] The prevalence of ESBLs and MBL among clinical isolates varies greatly worldwide and they are rapidly changing over time.

The present study was conducted in the Microbiology Laboratory of a tertiary care teaching hospital in Assam, North-East India between September 2017 and January 2018.

In the present study, 14.75% of the Gram negative bacteria were detected as ESBL producer. Production of ESBL among different organisms varies from 19.8-43% in various Indian studies[18-21] and from 16.07-21% in International studies.[22,23] The only β-lactams which are active against ESBL producers are 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, 17.11% isolates were found to be MBL producer which is in concordance with a study done in Karnataka[19] and higher than studies done in Maharashtra, India[24] and Nigeria.[23] Studies done in various parts of India has reported MBL prevalence between 2.9% to 41.2%.[19,20,24,25] High prevalence of MBL in some studies may be due to inclusion of only ICU patients while our study included all indoor, outdoor and ICU patients.

It has been proved that the prevalence of the ESBLs and MBLs among the clinical isolates varies from country to country and institution to institution within the same country. The low prevalence of the ESBL and MBL producers in our study could be due to the differences in the geographical distribution, which may have produced variations in the prevalence of the β-lactamases in different organisms, which may have given rise to the varied resistance patterns. Variations in the prevalence of beta-lactamases may be due to differences in selection of antibiotic,

local antibiotic and prescribing habits which differ in different institutions, states or countries.

In the present study, *Klebsiella spp.* Was found to be the most common ESBL producer which is in concordance with a study by Ibadin EE et al in Nigeria.[23] Most common MBL producer was found to be *Klebsiella spp* in our study as comparable with various other studies.[19-21] Several studies done in India have reported varying prevalence of different ESBL and MBL producer organisms among Gram negative isolates.[18-21,24] This discrepancy in the isolation rate may be due to varying prevalence of infection causing bacteria among various hospitals and even between different sites of infection.

Although ESBL and MBL producers were isolated from different specimen types, majority of them were isolated from urine specimen (54% and 51.7% respectively). This is in concordance with studies by Kaur N et al[19] and Ibadin EE et al.[23] Indiscriminate use of cephalosporins and their over the counter availability for treating UTI may have led to increasing resistance of bacteria isolated from urine.

In our study, no significant difference was seen in isolation rate of ESBL among indoor and outdoor patients. However, 15.6% ESBL producers were from outdoor patients representing community-acquired infection. This shows that ESBL production amongst the isolates from community-acquired infection is not uncommon and is in agreement with other studies.[22,26]

High percentage of ESBL producing isolates in community dwellers is a matter of concern. On the other hand, majority of MBL producing isolates were from indoor patients ($p<0.0001$) similar to several other studies.[19,23,24]

Analysis of data shows that beta lactamase producers are highly resistant to non-beta lactam groups of antibiotic like aminoglycosides, quinolones and trimethoprim/sulfamethoxazole. It has been seen that ESBL producers show resistance to other non-beta lactam group of antibiotics since the plasmid carrying the genes which encode for ESBLs also carries genes encoding resistance to other non-beta lactam antibiotics.[27] Similarly MBL production besides conferring resistance to carbapenems are also associated with aminoglycoside and flouroquinolone resistance.[28] The detection of these enzymes is a matter of great concern as treatment option for such isolates are very limited.

CONCLUSIONS

Infections with strains expressing ESBL or MBL are a challenge for both microbiologists and clinicians as they are having less therapeutic options. Early detection of these beta-lactamase producing isolates in a routine laboratory can help to avoid treatment failure. It can also help in providing an appropriate antimicrobial therapy and in avoiding the development and the dissemination of these multidrug resistant strains. Furthermore, strict antibiotic policies and measures to limit the indiscriminative use of cephalosporins and carbapenems in the hospital environment and to specify the indications for use or cycling classes of antibiotics are some of the options that can limit the selective pressure on nosocomial flora preventing emergence of drug resistance.

LIMITATIONS

Our study is limited by the fact that molecular analysis and characterization of ESBL and MBL types were not done.

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Source of Support: Nil. **Conflict of Interest:** None Declared.

Cite this article as: Binita Bhuyan, Pallabi Sargiary, Reema Nath. Study of Extended Spectrum Beta Lactamase and Metallo Beta Lactamase Production among Gram Negative Clinical Isolates from a Tertiary Care Hospital, North-East India. Int J Med Res Prof. 2018 July; 4(4):64-68. DOI:10.21276/ijmrp.2018.4.4.016