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Original Research Article

Study of Metallo-Beta-Lactamase Producing Gram Negative Bacteria in a Tertiary Care Hospital

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Abstract

Resistant bacteria are emerging worldwide as a threat to the favorable outcome of common infection in community & hospital settings. Beta-lactams remain a cornerstone for antimicrobial chemotherapy of a large number of bacterial infections. The most common cause of bacterial resistance to β- lactam antibiotics is the production of β- lactamases, followed by ESBL's and then the emergence of MBL activity which is one of the most feared resistance mechanism, because of its ability to hydrolyze virtually all β- lactams, including cabapenems. However MBL's are unable to hydrolyze monobactams & are not inactivated by β- lactamase inhibitors like clavulinic acid, sulbactam and Tazobactam. In any nosocomial settings, carbapenems are used as the last resort for treatment of MDR gram negative bacterial infections. MBL producing gram negative bacteria often exhibit resistance to additional classes of drugs and behave as multidrug resistant bacteria. Hence the present study was undertaken for detection of MBL producing gram negative bacilli and to help treating physicians to select appropriate antibiotic in our hospital. It was a prospective study conducted from April 2018 to July 2018 after IEC clearance. The Gram Negative isolates were first screened for MBL production with ceftazidime disc, & were further tested by Combined Disc Test Method (CDT) and Modified Hodge Test (MHT). Total 300 gram negative isolates were studied. In these, the MBL producers were 43.6%, majority of the isolates were from pus (19.3%), followed by urine (14.3%). Amongst these, *E. coli* was the most common organism isolated (16.3%), followed by *Klebsiella pneumoniae*(11%) and *Pseudomonas aeruginosa* (6.6%).By combined disc test a total of 131 MBL producing strains were isolated and 126 strains were detected by MHT. The exposure of bacterial strains to a multitude of β- lactams has induced mutation of β- lactamase in many bacteria, expanding their activity even against carbapenems, by the production of MBL resulting into fewer therapeutic alternatives. Hence detection of MBL is very important in respect to the treatment plan and sparing use of antibiotics to avoid their spread in the hospitals.

Keywords: Metallobetalactamase (MBL), Gram Negative bacteria (GNB), Carbapenem.

INTRODUCTION

Globally, resistant bacteria are emerging as threat to the favourable outcome of variety of common infections in community as well as in hospital settings. The most common cause of bacterial resistance to beta lactam antibiotics is the production of beta lactamases. The scenario is further complicated with evolution of extended spectrum beta lactamases. This is followed by emergence of Metallobetalactamase (MBL) activity which is one of the most feared resistance mechanisms because of its ability to hydrolyze virtually all betalactams, including carbapenems [1]. However MBL's are unable to hydrolyze monobactams. Eg: aztreonam. These strains are not susceptible to therapeutic serine β lactamase inhibitors (such as clavulanate and sulphones) [2].These genes are carried on highly mobile elements and allow their easy dissemination [3]. They can be transferred horizontally via plasmids or are chromosomally mediated and hence are rapidly spread to other bacteria [4].

Various molecular studies, show that carbapenmases i.e. enzymes hydrolyzing carbapenems are classified into four groups A,B,C and D. Metallobetalactamase belong to Ambler class B type of betalactamase. They have action on broad spectrum of substrates including penicillins, cephalosporins and carbapenems [1].Due to the frequent use of carbapenems there is an alarming increase in the carbapenem resistant strains due to selection pressure.

Production of MBL's in clinical isolates represents a serious therapeutic challenge. The detection of MBL – producing Gram negative bacilli is crucial to control the spread of resistance and for the optimal treatment of patients. It is mainly important in treating the critically ill and hospitalized patients [1,5].

MBL genes were first detected in *Pseudomonas aeruginosa*, But now they have spread to other members of Enterobacteriaceae also [6]. Notably increased morbidity and mortality rates have been observed in the critically ill patients [7, 8]. Hence we intend to detect the MBL producing gram negative bacilli in our hospital. MBLs hydrolyze all beta lactam antibiotics including carbapenem, with the exception of aztreonam [9]. They require zinc for their catalytic activity. Their activity is inhibited by metal chelators such as EDTA and Thiol compounds. The occurrence of an MBL – positive isolate in a hospital setting poses a therapeutic problem because the only therapeutic alternatives remaining are the Polymyxin B and Colistin [10]. Hence accurate identification and timely reporting of MBL producing bacteria will aid in preventing the spread of these multidrug resistant isolates. Hence the present study was conducted with the aim of studying, the gram negative isolates for the resistance to Ceftazidime by using Kirby-Bauer disc diffusion method for the possible presence of (screening) Metallo-Beta-Lactamase (MBL)**,** and to detect production of MBL in the above isolates phenotypically by combined disc method and Modified Hodge test, and to compare the results of both.

MATERIAL & METHODS

This study was conducted over a period of 4 months (April 2018-July 2018), after IEC clearance. This was a prospective study conducted at Department of Microbiology, Bharati Vidyapeeth (Deemed to be University) Medical College and Hospital, Sangli. A total of 300 non duplicate gram negative bacteria isolated from various clinical samples were included in the study. These isolates were screened for MBL production by using Kirby-Bauer disc diffusion method, using Ceftazidime disc (30µg).When the zone of inhibition for Ceftazidime is less than 18 mm, it is considered for MBL production [1].

The screened isolates were further tested phenotypically by Modified Hodge Test (MHT) and Combined Disc method (CDT).

Combined Disc method

Two Imipenem (IPM) discs (10 µg), one containing 10 µl of 0.5M (750 µg) anhydrous ethylenediamine-tetraacetic acid(EDTA)and the other without EDTA were placed 25 mm apart (center to center). An increase in zone diameter of ≥7mm around the IPM-EDTA disc compared to that of the IPM disc alone was considered positive for MBL [1]**.**

Modified Hodge Test

A 0.5 McFarland standard suspension of *E.coli* ATCC 25922 was prepared in 5 ml peptone water and diluted 1:10 by adding 0.5 ml of the o.5 McFarland to 4.5 ml of peptone water. A lawn of the 1:10 dilution of *E.coli* ATCC 25922 was prepared on a Muller Hinton Agar plate as for the routine disc diffusion test. The plate was allowed to dry for 3 to 10 minutes. A 10µg Imipenem disc was placed in the centre of the test plate and the test organism was streaked in a straight line from the edge of the disc to the edge of the plate. Four organisms were tested on the same plate with one drug. The plate was incubated at 37 ^{0}C in ambient air for 16-24 hours. After incubation, a positive MHT test was indicated by a clover leaf like indentation of the *E.coli* ATCC 25922 growing along the test organism growth streak within the disc diffusion zone and a negative MHT test was indicated by no growth of the *E.coli* ATCC 25922 along the test organism growth streak within the disc diffusion zone [9]. The mechanism behind is, inactivation of a carbapenem by carbapenemase producing strains enabling carbapenem susceptible indicator strain to extend growth towards a carbapenem disc, along the streak of inoculums of the tested strain [11].

The isolates were identified by standard microbiological methods and antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method according to CLSI guidelines [12].

An isolate was considered as MDR, if resistance was encountered to atleast 3 of the following classes of antimicrobial agents: β lactams, carbapenems, aminoglycosides & fluroquinolones & extremely drug resistant if found to be resistant to all the 4 classes of antimicrobial agents [13].

RESULTS AND DISCUSSION

Table-1: Organism wise distribution of MBL producers

Organism	Total no of isolates tested	No of MBL positive isolates	Percentage (%) of MBL out of total No of Gram Negative isolates (300)
Pseudomonas aeruginosa	47	20	6.6 %
Acinetobacter spp.	38	13	4.3%
Esch.coli	95	49	16.3%
K.pneumoniae	79	33	11%
Citrobacter spp.	11	06	02%
Proteus spp.	13	03	01%
Others	17	07	2.3%
Total	300	131	43.6%

Table-2: Sample wise distribution of clinical isolates of MBL producers.

Sample	Total Isolates	MBL Producer	% of MBL Producer out of total No of (300) isolates N= (300)
Blood	31	15	05 %
Pus	96	58	19.3 %
Urine	83	43	14.3 %
Fluid	08	05	01.6 %
Others	82	10	3.3 %
Total	300	131	43.6 %

Table-3: Comparison of Combined Disc method and Modified Hodge Test

Organism	Total	No of isolates resistant to Ceftazidime by Kirby Bauer disc diffusion method	Isolates positive by Combined disc method	Isolates positive by Modified Hodge test
Pseudomonas aeruginosa	47	25	20	19
Acinetobacter spp	38	16	13	12
E.coli	95	62	49	47
K.pneumoniae	79	41	33	33
Citrobacter spp.	11	8	06	06
Proteus spp.	13	4	03	03
Others	17	8	07	06
Total	300	164	131	126

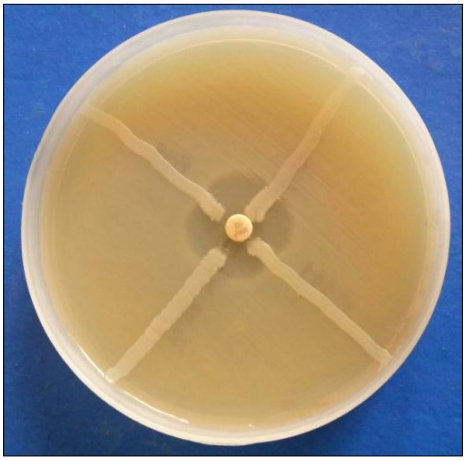

Fig-1: Modified Hodge Test (MHT)

In the present study, a total of 300 Gram negative bacteria were isolated during the study period. Out of those, 164 isolates showed resistance to Ceftazidime (30 µg) by disc diffusion method, which we have used as a screening method for MBL. Ceftazidime resistance is more significant in the case of enterobactericae where MBL producing strains can have low MIC for carbapenems & may appear sensitive on disc diffusion, as reported in different studies [6]. The sensitive or resistant pattern to IPM (10µg) / MRP (10µg) was not considered for MBL production as bacteria might harbour hidden MBL. So to ascertain that not even a single isolate carrying hidden MBL is missed, we used ceftazidime resistance as a screening tool [1]. Currently no standardized method for MBL detection has been proposed and PCR which is a gold standard is costly and not commonly available.

We have further used combined disc test & Modified Hodge test for detection of MBL production, In the view of finding the sensitive method for detection of MBL producers in GNB.

Out of total 300 gram negative isolates, 164 (54.6%) isolates were resistant to ceftazidime, which were considered as possible MBL producers. Out of these, 131 (43.6%) isolates showed MBL production. In our study all 131 strains were positive by CDT, but only 126 strains were positive by MHT. Panchal *et al* also found that CDT is a sensitive method for detection of MBL in GNB[14].

Various studies have stated varying rates of MBL production in Enterobactericae ranging from 2.9%(Deshmukh) *et al* [15] to 62%(Pandurangan et al) [16]We found 43.6% MBL producers in our study, which is similar to Chakraborty D *et al*. showing 41.2% MBL producing bacteria[17]. Out of those, 19.3% isolates were from pus followed by 14.3% isolates from urine and 5% from blood .Similar findings were noted by Bora *et al*[9]. Out of 131 MBL isolates highest were E. *coli* 49 (16.3%), followed by *Klebsiella pneumonia* 33(11%), *Pseudomonas aeruginosa* 20(6.6%) and Acinetobacter spp. 13(4.3%). Similar observations for MBL in GNB, were noted by Panchal *et al* [13], Anuradha C *et al* [4].

A large diverse group of betalactamases are disseminating on mobile genetic elements in clinically important gram negative organisms. This limits options for life threatening infections [1]. The infections with these organisms are associated with higher rate of mortality, morbidity and treatment costs [18]. Carbapenems are used as last resort for treatment of MDR gram negative bacterial infections in most of the hospital settings. However acquired resistance to this life saving antimicrobial is increasingly reported in Pseudomonas, Acinetobacter species & also in large numbers of members of Enterobactericae (mainly *E.coli* and Klebsiella) [1].

Antibiotic resistance pattern among MBL producing & non-producing bacteria is different MBL positive bacteria are showing more resistance to different classes of antibiotics due to formation of different types of antibiotic inactivating enzymes, thus mostly showing multidrug resistant pattern [15]. It is a challenge to the microbiology laboratories to detect emerging MBL producing GNB as there are no standardized guidelines available to detect them [2]. It is crucial to limit the spread of MBL gene in between bacteria as well as to start early appropriate treatment. In our study we have isolated total 164 Ceftazidime resistant strains. Further these strains were tested by CDT & MHT .Total 131 strains were positive by CDT, and 126 strains were positive by MHT. The MBL activity can be detected by both phenotypic & genotypic methods. Different studies have used different methods for detection of MBL strains according to their feasibility .PCR being accurate and sensitive is an ideal method for detection of MBL, giving reliable & satisfactory result, however the higher cost limits its use in routine diagnostic microbiological laboratory [2]. Hence a simple & inexpensive method is necessary. Therefore phenotypic methods are routinely employed, being simple, reliable, economical and sensitive. Imipenem –EDTA combined disc test is a simple test and can be used in routine diagnostic laboratories where molecular diagnostic techniques are not available. MBL positive isolates, show resistance to all β lactam antibiotics, aminoglycosides, tetracyclines and fluroquinolones [19]. In the present study, all the strains were sensitive to Tigecycline, Polymyxin B & Colistin. None of them were sensitive to Piperacillin Tazobactam.

Polymyxin & Colistin are peptide antibiotics & are presently used as last resort in MBL producing strains .However the high incidence of neurotoxicity & nephrotoxicity which is associated with these molecules limits its use [6].

CONCLUSION

Present study shows that significant numbers of MBL producing gram negative organisms (43.6 %) are isolated from our area. It is a serious epidemiological & therapeutic threat [1]. Hence there is a need of active surveillance and detection of MBL producers. Early detection of these will be helpful in respect to the treatment plan, judicious use of antibiotics and to avoid their spread in the other gram negative isolates in the hospital settings .It will also help to take proper preventive measures, create an effective antibiotic stewardship policy and reduce the hospitalization cost and stay. Hence early detection of MBL producers and incorporation of strict infection control practices are the best defense mechanisms which must be incorporated in our routine to reduce

mortality & morbidity due to these strains, giving best clinical outcomes.

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