Original Research Paper

Prevalence of Metallo-b-lactamase Producing Gram Negative Bacteria in a Tertiary Care Hospital, Patiala

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Abstract: The metallo-β-lactamases (MBLs) in gram negative bacteria have emerged as a major cause of health care associated infections. They hydrolyze all beta-lactam antibiotics including extended-spectrum cephalosporins and carbapenems, not inhibited by serine beta-lactamase inhibitors like clavulinic acid, sulbactum, and tazobactum and are resistant to many antibiotics. This study was undertaken to ascertain the prevalence of MBL producing gram negative bacteria. Out of 1546 culture positive gram negative isolates, 398 isolateswere multi drug resistant. These isolates were screened for carbapenem resistance by modified hodge test. Isolates were also checked for metallo- -lacatmase (MBLs) production by the EDTA combined disk test (CDT). MBLs - activity was detected in 43 (10.8%) isolates by CDT. In Multidrug resistance isolates,colistin being the most active agent. Emergence of MBL- producing pathogens in our setting creates an important challenge for clinicians and hospital epidemiologists, because it is added to the already high burden of antimicrobial.

Key Words: Modified Hodge test, imipenem, metallo-β-lactamases, non-fermenting bacilli

Introduction

The increase in the rates of antibiotic resistance is a major cause for concern in infections caused by gram negative bacilli. Mostly carbapenems, are used for the treatment of serious infections caused by extended spectrum-β-lactamase (ESBL) producinggram negative bacilli particularly for the members of family Enterobacteriaceae and non-fermenters, like Pseudomonas spp. and Acinetobacter spp. [1]

Organisms which produce Metallo-β-lactamases (MBL), have recently emerged as major health problem as they have the capacity to hydrolyze all β-lactams, including carbapenems. Such strains are not susceptible to even β-lactamase inhibitors (such as clavulanate and sulfones). MBL genes can be chromosome or plasmid mediated, and are often located in integrons as gene cassettes and these genes are carried on highly mobile elements, which help in easy dissemination. Transmissible MBLs were first described in Pseudomonas aeruginosa in Asia in the 1980s. [2] In recent years, MBL genes have spread from *Ps. aeruginosa* to members of the family Enterobacteriaceae. Infections with MBL-producing isolates are associated with a high morbidity and mortality. [3] The presence of an MBL-positive isolate in a hospital environment poses not only a therapeutic problem but is also a serious concern for infection control management. Treatment of these infections is particularly worrisome as the carbapenems are often agents of the last resort for resistant Gram-negative infections. [4,5]

With the global increase in the occurrence and types of MBLs, early detection is crucial, the benefits of which include timely implementation of strict infection control practices and treatment with alternative antimicrobials.[5] Molecular techniques are available to detect MBL producers. But,these are not available at every tertiary care hospital. Among the simple and cheaper methods available for testing MBL production is the imipenem (IMP)-EDTA combined disc test which is sensitive and specific.[8]

Objectives:

This study was undertaken to detect the prevalence of prevalence of Metallo-β-lactamase-

producing gram negative bacteria in clinical samples obtained from patients admitted in the tertiary care hospital.

Material and methods:

This study was carried out in the Departments of Microbiology the period from Jan 2016to June2016. Clinical samples were collected from patients admitted in the Hospital. Specimens, such as wound swabs, pus, blood and urine were included in the study. Samples were collected after obtaining informed oral consent from the patients. These isolates were studied for detection of prevalence of MBL production including their antibiogram. These samples were inoculated on blood agar and MacConkey agar and incubated at 37°C for 18–24 h under aerobic conditions. Appropriate biochemical tests were done to identify the organisms isolated. Antibiotic susceptibility test was performed with the help of the Kirby–Bauer disc diffusion method using commercially available discs on Mueller–Hinton agar. Interpretation was done according to the Clinical and Laboratory Standards Institute (2011) guidelines.*Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as control strains. Isolates of *P. aeruginosa* intermediate or resistant to at least three drugs in the following classes: beta-lactams, carbapenems, aminoglycosides and fluoroquinolones were labelled as multidrug-resistant *P. aeruginosa* (MDRPA). Moreover, isolates of *A. baumannii* resistant to at least two specific representatives of at least two classes of antibiotic categories: aminoglycosides, antipseudomonal penicillins, carbapenems, 3rd or 4th generation cephalosporins and fluoroquinolones were labelled as multidrug-resistant *A. baumannii* (MDRAB).Detection of MBL production was done by *Modified Hodge Test (MHT) and* Imipenem (IMP)-EDTA Combined Disk Test (CDT).[9]

Results:

Out of 10,689 clinical samples, culture was positive in 2154 samples, out which 1546 were gram negative isolates. Out of 1546, 398 gram negative isolates were multi drug resistant.All 398 isolates showed distorted carbapenem inhibition zones, indicating production of MBLs. These organisms were resistant tocephalosporins, aminoglycosides, monobactams, quinolones, piperacillin-tazobactum combination.

Out of 398 MDR, imipenem resistance was observed in 43 gram negative isolates (10.8%). All imipenem resistant strains (n = 43) were screened for MBL productionby *Modified Hodge Test (MHT) and* Imipenem (IMP)-EDTA Combined Disk Test (CDT). Location-wise distribution of MBL shows that 24 (55.8%) isolates were from the ICU, 19 (44.2%) isolates were from the post-operative patient (Table-1).

Of 43 MBL-positive isolates, 13 (30.2%), 12 (27.9%), 11 (25.5%), 3 (6.9%), 2 (4.6%) and 2 (4.6%) were recovered from urine, sputum, pus, catheter tip, blood and tracheal tubes respectively. The majority of *A. baumannii* isolates were recovered from respiratory tract specimens. Gram negative bacilli belonging to the family *Enterobacteriaceae* and *P. aeruginosa* were recovered from urine (Table 2).

All forty three isolates came out to (100%)MBL producers. Out of 43MBL isolates, 15 (34.8%) were Pseudomonas aeruginosa, 10 (23.3%) Acinetobacter baumannii,10 (23,3%) K.pneumoniae and 8 (18.6%) Esch.coli(Table 3).

Table 1. Location wise distribution of MBL producing isolates

Name of the ward	Number	%age
ICU	24	55.8%
Surgery	9	20.9%
Ortho	8	18.7%
ENT	2	4.6%
Total	43	100%

Table 2. Sample wise distribution of MBL producing isolates

Name of the ward	Number	%age
Urine	13	30.3%
Sputum	12	27.9%
Pus	11	25.7%
Catheter tip	3	6.9%
Blood	2	4.6%
Tracheal tubes	2	4.6%
Total	43	100%

Table 3 : Prevalence of MBL producing isolates in different bacteria

Name of the Isolate	Number	%age
Ps.aeruginosa	15	34.8%
Acinetobacter baumannii	10	23.3%
Esch.coli	10	23.3%
Klebsiella pneumoniae	8	18.6%
Total	43	100%

Table 4: The Antimicrobial sensitivity Pattern (%Age) Of 43 MBLs Isolated From various clinical sample Samples

Name of the Antibiotic	*Pseudomonas aeruginosa N=15*	*Acinetobacter baumannii N=10*	*Esch.coli N=10*	*Klebsiella pneumoniae N=8*
Amoxy clav	0	0	0	0
Gentamicin	2	1	2	2
Ciprofloxacin	0	0	1	0
Amikacin	2	1	2	2
Piperacillin – Tazobactum	1	0	3	1
Imipenem	0	0	0	0
Meropenem	0	0	0	0
Co-trimoxazole	0	0	0	0
Ploymyxin-B	15	10	10	8
Colistin	15	10	10	8

Discussion:

The increase in the antibiotic resistance is a major cause for concern in both non-fermenting gram negative bacilli and isolates of the family Enterobacteriaceae. β-lactams drugs have been the mainstay of treatment for serious infections. Metallo-β-lactamases (MBL) have recently acquired as one of the most worrisome resistance mechanisms owing to their capacity to hydrolyze all β-lactams, including carbapenems.

In our study,the prevalence rate of MBL-producing gram negative bacteria was 43 (10.8%), of which 58.1% were non- fermenters (Ps.aeruginosa and Acinetobacter baumannii) and 41.8% belong to familyEnterobacteriaceae (K.pneumoniae and Esch.coli).All other studiesalso reported MBL production ranging from 2.9% to 12%.[10,11,12,13,14] Comparison between modified Hodge test and DDST in our study revealed that DDST was more sensitive for detecting MBL. The same observation was reported by Jesudason *et al* [19]

The majority of these MBL isolates were from patients admitted in ICU ward (55.8%) and (44.2%) post-operative wards. Use of indwelling medical devices is common in these areas, which can play an important role in the spread of infective agents. These results simulated those of Nandy et al who reported 41.1% MBL producers from the ICU, 29.41% from surgical wards, 11.76%. [17]

In present study, out of 43 MBL-positive isolates, 13 (30.2%), 12 (27.9%), 11 (25.5%), 3 (6.9%), 2 (4.6%) and 2 (4.6%) were recovered from urine, sputum, pus, catheter tip, blood and tracheal tubes respectively. This correlates with the study by Attal et al and Hisaaki Nishio et al.[11,18]

These isolates were MDR resistant i.e they were resistant to all penicillins and cephalosporins (including inhibitor combinations), fluroquinolones, aminoglycosides and carbap enems.The presence of an MBL positive isolate in a hospital environment poses not only a therapeutic problem but is also a serious concern for infection control management. As a result of being difficult to detect and treat, such organism pose significant risks, particularly due totheir ability to participate in horizontal MBL gene transfer with other pathogens in the hospital. Therefore, use of carbapenems should be restricted to severe infections, especially in critically ill ICU patients, to avoid rapid emergence of resistant strains.In our study, colistin and ploymyxin B turned out to be the most effective antimicrobial against MBL producing multi drug resistant isolates. [13,15,19] As our institute does not have a molecular set-up, we were not able to confirm these findings by the genotypic method, which is limitation in our study.

Conclusion:

Reports from various parts of the world showing emergence of MBL enzymes in gram negative bacilli is alarming, and reflects the excessive use of carbapenems. Therefore, early detection and prompt instillation of infection control measures is important to prevent further spread of MBLs to other gram negative rods. Additionally, it is also important to follow antibiotic restriction policies to avoid excessive use of carbapenems and other broad-spectrum antibiotics. The effective and highly sensitive phenotypic methods can be employed in any laboratory to both screen for and confirm the presence of this important mechanism of antimicrobial resistance. This will further help in timely implementation of strict infection control practices as well as clinical guidance regarding the potential risks for therapeutic failure.

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Conflict of Interest: None

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