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Research Paper

Comparison of Two Phenotypic Methods, Prevalence and Antimicrobial Susceptibility Profile of Metallo-B-Lactamases (M-B-L) Producing Gram Negative Bacilli

Medical Science

KEYWORDS :

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ABSTRACT

Introduction: Emergence of carbapenem resistance notably Metallo- beta- lactamase(MBLs) in Gram negative bacilli(GNB) is considered as a worldwide public health concern as there are few antibiotics in reserve beyond carbapenems. Therefore early detection of organisms producing carbapenemase in particular MBLs is necessary for timely implementation of strict infection control practice and treatment with alternative antimicrobials.

Objective: To know prevalence of Metallo- beta- lactamase production & comparision of two methods in various Gram negative bacilli.

Material & Methods: 1238 isolates of GNB were collected from various clinical specimens from different sites between June 2012 to July 2012 were subjected to antibiotic susceptibility testing by Kirby Bauer disk diffusion method as per Clinical & Laboratory Standards institute guidelines(CLSI). Imipenem resistant isolates were selected for detection of Metallo- beta- lactamase production by Imipenem-EDTA combined disk test (CDT) & Imipenem - EDTA double- disc synergy test (DDST).

Results: Out of 1238 GNB 144 imipenem resistant strains isolated, out of these 67 were positive for MBL by CDT & DDST. Commonest organisms isolated were pseudomonas aeruginosa, followed by Klebsiella spp & Acinetobacter spp. MBL were most common isolates in burns patients.

Conclusion: Our finding suggests that there is a need to do surveillance to detect MBL producer. Carbapenem needs to be used judiciously. CDT is the most sensitive method for detection of MBL production in GNB. There is variation in subjective interpretation of result in DDST.

INTRODUCTION:

The increase in the rates of antibiotic resistance is a major cause for concern in Gram negative bacilli. β-lactams have been the mainstay of treatment for serious infections.

Acquired metallo-β-lactamases (MBL) have recently emerged as one of the most worrisome resistance mechanisms owing to their capacity to hydrolyze all β-lactams, including carbapenems. Such strains are not susceptible to therapeutic serine β-lacatamase inhibitors (such as clavulanate and sulfones). Moreover, their genes are carried on highly mobile elements, allowing easy dissemination. [1]

The MBL activity can be detected by both phenotypic and genotypic methods. Different studies have used different methods according to their feasibility. Though the molecular methods are highly sensitive and specific, their use is limited only to the research laboratories. The phenotypic methods being simple, sensitive, economical and reliable, they can be routinely performed in microbiological laboratories. Several phenotypic methods are available for the detection of the MBLs which are produced by bacteria. Most of these methods are based on the ability of the metal chelator (EDTA) and the thiol based compounds to inhibit the enzyme activities. [2]

Five different types of MBLs whose prevalence are increasing rapidly are IMP, VIM, SPM, GIM and SIM.[3] Among these, IMP and VIM are the most predominant.[4] 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]

AIMS AND OBJECTIVES

1) To evaluate the accuracy of two different phenotypic methods to detect MBL production in gram negative bacilli.
2) To know prevalence of MBL production in various Gram negative bacilli.
3) To find out antibiotic sensitivity profile of MBL producing Gram negative bacilli.

MATERIALS AND METHODS:

2859 samples were collected during two month period from June & July 2012 in the department of Microbiology. The samples processed were: Urine, Pus, Wound swab, Sputum, Blood, Fluid, Endotracheal secretion and Tissue. Samples were cultured on Mac Conkey agar, Blood agar and Nutrient agar. A total of 1238 isolates of Gram Negative Bacilli were isolated. Identification was done by battery of biochemical tests.

Antimicrobial susceptibility of all the isolates was performed by the *Kirby Bauer* disc diffusion method according to the CLSI guidelines 2012.

(Table:1)The following Antibiotics were tested for

Pseudomonas aeruginosa	Enterobacteriaceae
CEFTAZIDIME (30µg)	AMPICILLIN–SULBACTUM(20 µg)
PIPERACILLIN(100µg)	CO-TRIMOXAZOLE(25µg)
PIPERACILLIN-TAZOBACTAM(110µg)	CEFUROXIME(30 µg)
CEFEPIME/ TAZOBACTM(40 µg)	CEFTRIAXONE(30 µg)
CEFOPARAZONE(75µg)	CHLORAMPHENICOL (30 µg)
NORFLOXACIN(5 µg)	CIPROFLOXACIN(5 µg)
TOBRAMYCIN(10 µg)	COLISTIN(10 µg)
LEVOFLOXACIN(10µg)	CEFOPARAZONE-SULBACTUM(105 µg)
POLYMIXIN B(300UNITS)	DOXYCYCLINE(30 µg)
GEMIFLOXACIN(5 µg)	GENTAMICIN(10 µg)
AZETREONAM(30 µg)	AMIKACIN (30 µg)
NETIMYCIN(30 µg)	GATIFLOXACIN(10 µg).
IMIPENEM (10 µg).	IMIPENEM (10 µg)

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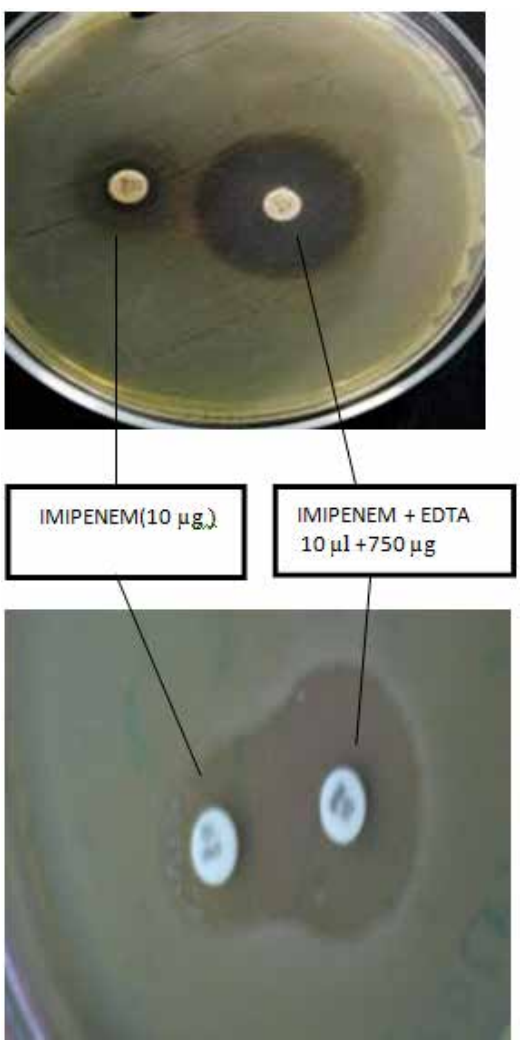
Metallo-β-lactamase screening:
MBL production was detected in imipenem-resistant isolates by phenotypic tests. The Imipenem (IMP)-EDTA combined disc test & Imipenem - EDTA double disc synergy test (DDST) were used.

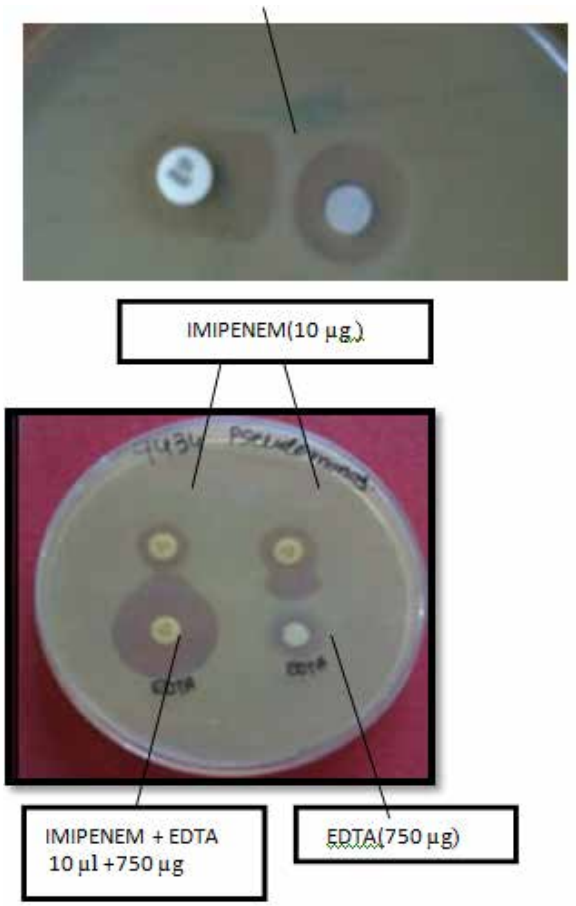
1. Imipenem- EDTA combined disc test (CDT).
The Imipenem- EDTA combined disc test (CDT) was preformed as described by yong et al. The test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI. A 0.5 M EDTA solution was prepared by dissolving 18.61 g. of EDTA in 100 ml of distilled water and adjusting its pH 8.0 by using NaOH. The Mixture was sterilized by autoclaving. Two imipenem (10µg) discs were placed on the surface of an agar plate at distance of 20 mm and 10 ul EDTA solution was added to one of them to obtain a desired concentration of 750 µg. The inhibition zones of imipenem and imipenem- EDTA discs were compared after 16 to 18 h of incubation in air at 37°C. In the combined disc test, if the increase in inhibition zone with the imipenem and imipenem- EDTA disc was ≥7 mm than the imipenem alone, it was considered as MBL positive.[6-9]

POSITIVE COMBINED DISC TEST
(FIG:1)



2. Imipenem - EDTA double- disc synergy test (DDST).
The IMP-EDTA double disc synergy test was performed as described by Lee at al. Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by CLSI. An imipenem (10 µg) disc was placed 20 mm centre to centre from a blank disc containing 10 µl of 0.5 M EDTA(750 µg). Enhancement of the zone of inhibition in the area between imipenem and the EDTA disc in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result. [10]

(FIG:2)
SYNERGISTIC ZONE OF INHIBITION BETWEEN THE DISC



RESULT:
Of the total 1238 Gram negative isolates, 144(11.63%) showed imipenem resistance in a period of 2 months by the disk diffusion method . A total of 67 (46.52%) isolates showed MBL production by the imipenem (IMP)-EDTA combined disc test & Imipenem - EDTA double disc synergy test (DDST).

TABEL 1: Organism wise prevalence of MBL PRODUCERS IN GNB

Organism	Imipenem Resistant	MBL Producer
Pseudomonas aeruginosa	51(35.4%)	25(37.3%)
Klebsiella pneumonia	34(23.6%)	21(31.3%)
Acinetobacter baumanii	30(20.8%)	11(16.4%)
E. coli	29(20%)	10(14.9%)

Organisms of MBL producer were mostly from *Pseudomonas aeruginosa followed by Klebsiella pneumonae and few Acinetobacter baumanii and Escherichia coli.*

TABEL 2: WARD wise prevalence of MBL PRODUCERS IN GNB

WARD	Imipenem Resistant	MBL Producer
Burns	49(34%)	27(40.2%)
Surgery	35(24.3%)	15(22.3%)
Orthopedic	27(18.8%)	13(19.4%)
Medicine	14(9.7%)	7(10.4%)
Pediatric	15(10.4%)	4(6%)
ENT	4(2.7%)	1(1.4%)

Total isolates of MBL producer were mostly encountered in burns ward then surgical and orthopaedic ward and few from medicine, paediatrics, ENT.

TABEL 3: Specimen wise prevalence of MBL PRODUCERS IN GNB

Specimen	Imipenem Resistant	MBL Producer
Swab	70(48.6%)	39(58.2%)
Urine	25(17.3%)	7(10.4%)
Endotracheal secretion	25(17.3%)	15(22.3%)
Blood	5(3.4%)	1(1.4%)
Fluids	9(6.25%)	2(3%)
Pus	4(2.7%)	0(0%)
Tissue	5(3.5%)	3(4.4%)
Sputum	1(0.7%)	0(0%)

Prevalence of MBL Producer is maximum in swab specimen followed by endotracheal secretion and urine and few in tissue, fluids & blood.

DISCUSSION:

Since there are no standard guidelines for detection of MBL, different studies have reported the use of different methods. PCR analysis is the gold standard method for the detection of MBL production, but it is not feasible in routine microbiology laboratory.

In present study, out of 1238 gram negative bacilli isolates, 144 Imipenem resistant isolates out of them 67 (46.53%) isolates were screening test positive for MBL production. Out of two methods used for confirmation of MBL production, CDT is the most sensitive method. Interpretation of DDST results is more subjective as it depends upon the microbiologist's expertise to discriminate true synergism from the intersection of inhibition zones.

Prevalence of MBL production, was found to be highest in *Pseudomonas* (37.31%)

Table 4: Antimicrobial susceptibility profile of MβL isolates n=67

Antibiotic disc	Pseudomonas aeruginosa n-25	Klebsiella pneumonia n=21	Escherichia coli n =10	Acinetobacter baumani n=11
Ceftazidime (30 ug)	00	-	-	--
Piperacillin-tazobactam (110ug)	11(44%)	-	-	-
Cefepime/ tazobactm (40 ug)	11(44%)	-	-	-
Cefoparazone (75 ug)	00	-	-	-
Norfloxacin (5 ug)	00	-	-	-
Tobramycin (10 u)	**00**	**-**	**-**	**-**
Levofloxacin (10 ug)	**00**	**-**		-
Polymixin B (300units)	25 (100%)	21 (100%)	10 (100%)	11 (100%)
Gemifloxacin (5 ug)	00	-	-	-
Azetreonam (30 ug)	8(32%)	-	-	-
Netimycin (30 ug)	8(32%)	-	-	-
Imipenem (10 ug)	00	00	00	00
Ampicillin –sulb actum (20 ug)	-	4(19.04%)	00	4(36.36%)
Co-trimoxazole (25 ug)	-	00	00	00
Cefuroxime (30 ug)	-	00	00	00
Ceftriaxone (30 ug)	-	00	00	00
Chloramphenicol(30 ug)	--	6	2(20%)	1(9.09%)
Ciprofloxacin(5 ug)	-	00	00	00
Colistin (10 ug)	25 (100%)	21 (100%)	10 (100%)	11 (100%)
Cefoparazone-sulbactum (105 ug)	-	4 (19.04%)	00	5 (45.45%)
Doxycycline (30 ug)	-	00	00	00
Gentamicin (10 ug)	-	1(4.7%)	00	3(27.27%)
Amikacin (30ug)	-	00	00	00
Gatifloxacin (10 ug).	-	6(28.57%)	1(10%)	3(27.27%)

In present study all isolates were sensitive to polymyxin B & Colistin.

Followed by *Klebsiella pneumonia*(31.34%), *Acinetobacter(*16.41%) and *E. Coli*(14.93%), but not in other gram negative bacilli. The majority of the organisms were from swab and endotracheal secretion samples and from patients of the burns ward and surgical ward; areas where the majority of critically ill patients are concentrated.

In our study burns patients were affected more than the surgical patients because of burns in a patients provide a suitable site for bacterial multiplication and are more persistent richer sources of infection than surgical wounds, mainly because of the larger area involved and longer duration of patient stay in the hospital .[11]

MBL positive isolates usually shows resistance to all β-lactam antibiotics, aminoglycosides, tetracycline, and fluoroquinolones.

However they remain sensitive to polymyxin B & Colistin.

It is similar to study carried out by Sarkar *et al*{2006}followed by 75% sensitivity to azetronam, 62.5% sensitivity to colistin while 50% sensitivity was shown to pipercillin/ tazobactem. So use of combination therapy of either polymyxin B or azetronam with either aminogylcosides or fluroquinolones should be used and never as monotherapy Yoon *et al* {2006}.[12,13]

CONCLUSION:

The early detection of MBL-producing isolates would be important for the reduction of mortality rates for patients infected with MBL producing isolates and also to avoid the intra hospital dissemination of such strains.

'Imipenem-EDTA combined disk test' (CDST-IPM) is the most sensitive method for detection of MBL production in gram negative bacilli. CDST-IPM is the method that can be used as a convenient screening method for detection of MBL production in Gram negative bacilli.

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