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Prevalence of Metallobetalactamase (MBL) in Nonfermenting Gram-Negative Bacilli (NFGNB) isolates at a tertiary care hospital in Assam, India

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Abstract

Introduction: With the advent of the usage of a new beta lactam group of antibiotics i.e., Carbapenems, heralded a new therapeutic option for the serious Gram negative infections, but soon emerged the enzymes, which could destroy even these Carbapenems and pan drug resistant strains began to emerge. Among them one of the notable is the Metallo-beta-lactamase (MBL) producing strains which is mostly found among non fermenting Gram negative bacilli (NFGNB).

Materials and methods: The present study was conducted to know the prevalence of Metallobetalactamase (MBL) among non- fermenting Gram-negative bacilli (NFGNB) isolates. A total of 200 samples were collected from various wards (Indoor) and outpatient departments (OPD) of our hospital from June 2011 to May 2012. Gram staining, culture was done for identification of nonfermenters upto species level. Antimicrobial susceptibility was done and detection of MBL was done in Imipenem resistant isolates by IMP-EDTA combined disk method.

Results: Out of the 200 NFGNB isolates, *Pseudomonas aeruginosa* was the common organism (86%), followed by *Acinetobacter baumannii* (3.5%) and *Pseudomonas fluorescens* (2.5%). 30 (15%) isolates were Imipenem resistant and 9 (4.5%) were MBL producers .*Pseudomonas aeruginosa* producing MBL was 5.2%.. Correlation between Imipenem resistance and MBL producer was found to be significant (p-value <0.000001). All the MBL isolates were 100% sensitive to Colistin and Polymyxin B while they were 100% resistant to Imipenem, Ceftazidime, Cefepime, Aztreonam and Ciprofloxacin.

Conclusions: This study underlines the unique problem of MBL mediated resistance in these non-fermenters, and emphasizes the importance of their isolation and identification.

Keywords: Metallobetalactamase, Nonfermenting GNB, Pseudomonas, Acinetobacter

Introduction

With the advent of the usage of a new beta lactam group of antibiotics i.e., carbapenems, heralded a new therapeutic option for the serious Gram negative infections, but soon emerged the enzymes, which could destroy even these carbapenems and pan drug resistant strains began to emerge [1].

Based on molecular studies, two types of carbapenem-hydrolyzing enzymes have been described. First types are the serine carbapenemases belonging to molecular Class A or D of Ambler. Plasmid mediated KPC enzymes and some OXA type beta lactamases which exhibit carbapenemase activity are included in this group only. The second types are the metallo-β-lactamases (MBLs), requiring divalent cations, usually zinc, as metal cofactors for enzyme activity [2]. Metallo beta lactamases (MBLs) are the Ambler class B beta lactamases which has the capacity to hydrolyze with the exception of aztreonam, all beta lactams including carbapenems[3].

Metallo-beta-lactamase (MBL) producing strains are found especially among non fermenting Gram-negative bacilli (NFGNB) like *Pseudomonas aeruginosa* and *Acinetobacter* species[4].Studies in India showed the prevalence of MBL in NFGNB to be from as low as 1.7% to as high as 62%[5,6].

Keeping these things in mind the present study was conducted to know the prevalence of MetalloBetalactamase (MBL) among nonfermenting Gram-negative bacilli (NFGNB) isolates.

Materials and methods

The study was conducted in the Department of Microbiology; Gauhati Medical College & Hospital over a period of 1year, from June 2011 to May 2012 after ethical clearance was obtained from the Institutional Ethical Committee.

A total of 200 samples were collected from various wards (Indoor) and outpatient departments (OPD) of our hospital. The samples were: Urine, Sputum, pus, wound swab, tracheal aspirate, blood, throat swab, aural swab, pleural fluid, foley's catheter tip and CSF.

A preliminary Gram staining of direct smear was performed and examined microscopically to determine the likely organism present according to the methods described by Duguid *et al* [7].

The samples were cultured on MacConkey agar and Blood agar and processed for identification of nonfermenters upto species level as per methods described by Collee *et al* [8].

Antimicrobial susceptibility of the nonfermenters was done by Kirby Bauer Disc diffusion method according to the CLSI guidelines [9]. Commercially available antibiotic discs were obtained from Hi Media Laboratories Limited, Mumbai. Antibiotics used Amikacin (30mcg), Aztreonam(30mcg), Cefepime(30mcg),Ceftazidime(30mcg),Ciprofloxacin (5mcg), Imipenem(10 mcg) ,Piperacillin-tazobactum(100 /10mcg), Colistin (10 mcg) and Polymyxin B(300Units). Detection of MBL was done in Imipenem resistant isolates by IMP-EDTA combined disk method as described by Yong D *et al* [10].

Results

A total of 200 nonfermenters isolated from different clinical samples and different age groups were identified and characterized. *Pseudomonas aeruginosa* was the common organism (86%), followed by *Acinetobacter baumannii* (3.5%) and *Pseudomonas fluorescens* (2.5%). Table 1 show the number and percentage of the various nonfermenters isolated obtained in the study. The numbers of male patients were 125 (62.5%) while female patients were 75 (37.5%). Majority of the patients were in the age group of 21-40 years. Table 2 shows the sex and age distribution of the 200 patients.

Majority of the patients in this study were inpatients, the highest number of 55(27.5%) samples were obtained from the Medicine Department. Table 3 shows the inpatient and outpatients distribution of the cases while Table 4 shows the source of samples.

Out of the 200 isolates, the maximum of 50 (25%) were isolated from urine followed by 48 (24%) sputum and 34 (17%) from pus.

Table 5 shows the various specimens from which NFGNB were isolated.

Out of the 200 NFGNB isolates, 30 (15%) were Imipenem resistant, among them 9 (4.5%) were Metalobetalactamase (MBL) producer. Correlation between Imipenem resistance and MBL producer was found to be significant (p-value <0.000001).

Pseudomonas aeruginosa producing MBL was 5.2%. Five (55.6%) of the isolates were from tracheal aspirate and 4 (44.4%) from urine. Table 6 shows the distribution of MBL producers.

All the MBL isolates were 100% sensitive to Colistin and Polymyxin B while they were 100% resistant to Imipenem, Ceftazidime, Cefepime, Aztreonam and Ciprofloxacin.

Table 7 shows the antibiotic susceptibility pattern of the isolates.

Table1: Various non-fermenters isolated from the clinical specimens.

ORGANISM ISOLATED	NUMBER	PERCENTAGE (%)
Pseudomonas aeruginosa	172	86
Acinetobacterbaumannii	7	3.5
Pseudomonas fluorescens	5	2.5
Pseudomonas alcaligenes	4	2
Pseudomonas putida	4	2
Stenotrophomonasmaltophilia	3	1.5
Burkholderiacepacia	3	1.5
Pseudomonas pseudoalcaligenes	1	0.5
Pseudomonas veronii	1	0.5
TOTAL	200	100

Table 2: Age and sex wise distribution of patients.

AGE (IN YEARS)	NO. OF PATIENTS		
	MALE	FEMALE	TOTAL
< 1	4	1	5
1 – 10	8	4	12
11 -20	9	12	21
21 - 30	20	28	48
31 - 40	23	14	37
41 - 50	22	8	30
51- 60	20	3	23
61 – 70	11	3	14
71 - 80	7	3	10
>81	1	0	1
Total	125 (62.5%)	75 (37.5%)	200 (100%)

Table 3: Inpatient / Outpatient wise distribution of cases.

Type	Number	Percentage (%)
Inpatient	156	78
Outpatient	44	22
TOTAL	200	100

Table 4: Source of samples collected.

Deptt/ Ward/ OPD	No. of samples	Percentage (%)
Medicine	55	27
Surgery	42	21.5
Urology	30	15
ICU	28	14
Burn ward	11	5.5
PICU &NICU	8	4
Plastic surgery	3	1.5
Orthopaedics	2	1
Dermatology	4	2
CTVS	2	1
T.B. & Chest	5	3
Obstetrics &Gynaecology	5	2.5
E.N.T	5	2.5
TOTAL	200	100

Table 5: Various specimens from which NFGNB were isolated.

SAMPLE	NO.OF CASES	PERCENTAGE (%)
Urine	50	25
Sputum	48	24
Pus	34	17
Wound swab	26	13
Tracheal aspirate	24	12
Blood	6	3
Throat swab	4	2
Aural swab	3	1.5
Pleural fluid	2	1
Foley's catheter	2	1
CSF	1	0.5
TOTAL	200	100

Table 6: Distribution of MBL producers.

Organisms	Total no. of isolates	Imipenem resistant	MBL production by IMP-EDTA combined disc test
Pseudomonas aeruginosa	172	26	9 (5.2%)
Acinetobacterbaumanii	7	0	0
Pseudomonas fluorescens	5	0	0
Pseudomonas alcaligenes	4	0	0
Pseudomonas putida	4	1	0
Stenotrophomonas maltophilia	3	2	0
Burkholderiacepacia	3	0	0
Pseudomonas pseudoalcaligenes	1	1	0
Pseudomonas veronii	1	0	0
Total	200	30(15%)	9 (4.5%)

Table 7: **Antibiotic susceptibility pattern of MBL producers.**

Antibiotic	Sensitive	Resistant
Colistin	9(100%)	Nil
Polymyxin B	9(100%)	Nil
Amikacin	1 (11.1%)	8 (88.8%)
Aztreonam	Nil	9 (100%)
Ceftazidime	Nil	8 (88.8%)
Cefepime	Nil	8 (88.8%)
Ciprofloxacin	Nil	9 (100%)
Piperacillin- tazobactum	1 (11.1%)	8 (88.8%)
Imipenem	Nil	9 (100%)

Discussions

In this study, *Pseudomonas aeruginosa* was the commonest NFGNB isolated (86%) which was similar to the studies of Kaushal ML *et al* [11] and Gokale SK *et al* [12] while a few studies had lesser isolation [13, 14,15,16,17].Table 8 shows the comparison of distribution of NFGNBs in various studies.

In the present study, the percentages of male patients were higher than females. Similar findings were reported by Yashodhara P *et al* [13] and Kaushal ML *et al* [11].

Highest numbers of cases in this study were isolated in the age group of 21-40 years and majority of the cases were adults. Similar observations were made by other workers Yashodhara P *et* al [13] and Kharangate NV *et al* [18].

In the present study, majority of the patients in this study were inpatients (78%) and maximum isolates were recovered from urine (25%), sputum (24%) and pus (17%). Studies by Mishra B *et al*[19]and Malini A *et al*[20] isolated most of the NFGNB from pus samples.

In the present study, 9 (4.5%) NFGNB isolates were MBL producers. A lower percentage of MBL (1.7%) was found by Deshmukh DG *et al* [21] in NFGNB while a higher percentage of 33.33% was found by De AS *et al*[22] and 62% by Jesudason MV *et al* [23]. Table 9 shows the comparison of MBL isolates in various studies.

Correlation between Imipenem resistance and MBL producer was found to be significant (P-value <0.000001). One study found 12% of *Pseudomonas* isolates that were resistant to imipenem, all were MBL producers [24].

In the present study, all MBL producers were 100% resistant to Imipenem, Ceftazidime, Cefepime, Aztreonam and Ciprofloxacin. This correlates with the study by Kumar SH *et al*[25] where all MBLs isolates were resistant to carbapenem, third generation cephalosporins, aminoglycosides and quinolones.

Table 8: Comparison of distribution of NFGNB in various studies.

STUDIES	PERCENTAGE								
	Pseudomonas aeruginosa	**Acinetobacter baumannii**	**Pseudomonas fluorescens**	**Pseudomonas alcaligenes**	**Pseudomonas putida**	**Stenotrophomonas maltophilia**	**Burkholderiacepacia**	**Pseudomonas pseudoalcaligenes**	**Pseudomonas veronii**
Kaushal ML *et al*. (1996)[11]	88.8	3.7	2.2	-	0.5	3.7	-	-	-
Yashodhara P *etal*. (1997)[13]	57	15	8	-	7	-	-	-	-
Veenu*et al*. (1999)[14]	72.66	3.66	3.66	-	3	-	-	-	
Arora U *et al*. (2003)[15]	72.83	8.4	2.4	-	0.4	1.6	-	-	-
Malini A *et al*. (2009)[16]	53.8	22.2	10.8	-	-	2.6	-	-	-
Gokale SK *et al*. (2012)[12]	82.3	15.4	-	-	-	-	-	-	-
Parandekar PK *et al*. (2012)[17]	69.8	18.9	1.7	-	-	4.3	3.4	-	-
Present study (2011-2012)	86	3.5	2.5	2	2	1.5	1.5	0.5	0.5

Table 9: Comparison of MBL isolates in various studies.

Studies	Year	Percentage (%)		
		Total MBL in NFGNB	***Pseudomonas aeruginosa***	***Acinetobacter* sp.**
Jesudason MV *et al.*[23]	2005	62	75	-
Mendiratta DK *et al*[26]	2005	8.6	8.6	
De AS *et al.*[21]	2010	33.33	28.57	36
Deshmukh DG et al.[22]	2011	1.7	36.8	21
Present study	2011-2012	4.5	5.2	-

Conclusion

Pseudomonas aerugionsa and *Acinetobacter* species are the most common nonfermenters associated with human infections. This study underlines the unique problem of MBL mediated resistance in these nonfermenters, which has created a therapeutic challenge for the clinicians and microbiologists. The fact that the nonfermenters are resistant to the commonly used antibiotics emphasizes the importance of including tests for their isolation and identification schemes. Many clinical laboratories are not fully aware of the importance of the MBL producers and of methods to detect them which has lead to their widespread dissemination. The consequence of this has been avoidable therapeutic failures (sometimes fatal) in patients who received inappropriate antibiotics and outbreaks of infections which were caused by multidrug-resistant gram negative pathogens that required expensive control efforts. Hence, their detection must be quick, for formulating an antibiotic policy and containment measures to solve the issue of antibiotic resistance.

Conflict of Interest: None

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