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Detection of Metallo-beta lactamase production in clinical isolates of Nonfermentative Gram negative bacilli

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Abstract: *The alarming increase in the frequency of MBLs presents an emerging threat of complete resistance to all therapeutic drugs in practice, leaving the potentially toxic polymyxin B and colistin as the only options among antibiotics.The aim of this study was to determine the prevalence of MBL producing strains among Pseudomonas and Acinetobacter species in our hospital setting and to evaluate the various phenotypic methods for detection of Metallo beta-lactamases. All clinical samples were cultured on blood agar and Mac Conkey's agar and the Acinetobacter and Pseudomonas species were identified by standard bacteriological techniques. The Imipenam resistant strains were subjected to MBL detection by Modified Hodge test, Double Disk Synergy test and the Disk Potentiation test. 15.3% Pseudomonas strains and 43.5% Acinetobacter strains were resistant to Imipenem. Among them 46.7% of Pseudomonas isolates and 70% of Acinetobacter isolates were positive for carbapenemase production by Modified Hodge test. Only 6.7% of Imipenem resistant Pseudomonas and Acinetobacter isolates were metallo beta lactamase [MBL] producers. Modified Hodge test can be used as a screening test for detecting carbapenemase production. Both double disk synergy test and disk potentiating tests are equally effective in the detection of MBL production.*

Key words: *Acinetobacter, Carbapenemase, Metallo-beta-lactamase, Pseudomonas*

I. Introduction

The first strain producing the Metallo-beta-lactamase(MBL) i.e., Pseudomonas aeruginosa was isolated in Japan in 1988 [1]. Since then, the presence and spread of MBL positive strains have been observed world over, India being no exception. Increased mortality rates have been documented for patients infected with MBL-producing Pseudomonas aeruginosa, especially due to inadequate empirical therapy.

Acinetobacter baumanii, a major nosocomial pathogen, is a matter of great concern due to its intrinsic and acquired resistance mechanisms. [2] Horizontal transfer of these MBL producing genes occur and resistance disseminates to other gram negative bacteria. Genes coding for MBL reside in mobile gene cassettes inserted in the integrons. [3] Intensive care units of hospitals are the epicenters for MBL-positive strain isolations. These are the commonest agents of ventilator associated pneumonia. But sadly now, studies show that isolates from outside the intensive care units are also developing multi drug resistance due to MBL production. [4]

Carbapenems had been the drug of choice for the infections caused by most penicillin- or cephalosporin-resistant gram negative bacteria due to its broad spectrum activity and stability to hydrolysis by most beta-lactamases. This does not hold good anymore due to rapid uprise of MBL producing strains. These strains have the capacity to produce hydrolyzing enzymes against virtually all classes of beta-lactams.

MBL can hydrolyze β-lactams from all classes except the monobactams.[5] The alarming increase in the frequency of MBLs presents an emerging threat of complete resistance to all therapeutic drugs in practice, leaving the potentially toxic polymyxin B and colistin as the only options among antibiotics.[6] Thus, their continued spread would be a clinical disaster. The relentless threat posed by microbial drug resistance has achieved the dimension of a global pandemic. The benefits of early detection, which is crucial, include timely implementation of strict infection control practices to prevent further intra- and inter-hospital spread, and treatment with alternative antimicrobials.

It is now clear that with these two gram-negative pathogens we are closer to the "end of antibiotics" than with any other gram-negative or gram-positive bacterial pathogens. [7] Therefore, it is essential to rapidly screen and detect MBLs, which could help in modifying therapy and initiate effective infection control to prevent further dissemination. This is a measure that has to be taken urgently because the pan-resistant bacteria are taking a toll of lives throughout the globe and turning back the clock to the pre-antibiotic era.

Hence we undertook this study to detect the production of Metallo beta-lactamase(MBL) in clinical isolates of nonfermentative Gram-negative bacteria and also to evaluate the various phenotypic methods for detection of Metallo beta-lactamases.

II. Materials And Methods

A prospective study was conducted over a period of 6 months at the department of Microbiology. 850 urine samples, 845 blood culture samples, 547 pus samples, 243 sputum samples, 38 throat swabs and 25 endotracheal tube suction samples were collected taking aseptic precautions from patients admitted in various wards during the study period. Only those samples which yielded the growth of Pseudomonas and Acinetobacter were included in the study and samples which yielded organisms other than Pseudomonas and Acinetobacter were excluded from the study.

All clinical samples were cultured on blood agar and Mac Conkey's agar and incubated aerobically at 37ºC overnight. The Acinetobacter species and Pseudomonas species were identified by standard bacteriological techniques. [3] Antimicrobial sensitivity testing was performed on Mueller Hinton agar by Kirby Bauer disc diffusion method using amikacin 30 µg, ceftazidime 30 µg, ceftriaxone 30 µg, ciprofloxacin 5µg, imipenem 10µg, chloramphenicol 30µg and piperacillin/tazobactum 100/10µg discs and interpreted as per CLSI recommendations.[8] Pseudomonas aeruginosa ATCC 27853 strain was used as control. The Imepenam resistant strains were subjected to MBL detection by Modified Hodge test, Double Disk Synergy test and the Disk Potentiation test.

Modified Hodge Test: A lawn culture of overnight suspension of E.coli ATCC 25922 adjusted to 0.5 McFarland Standard was inoculated using sterile cotton swab onto Mueller-Hilton agar. A 10 µg Imipenem disk was placed at the centre of the plate and the test strain was streaked from edge of the disk to the periphery of the plate in four different directions. The plate was incubated overnight aerobically at 37°C. The presence of a 'cloverleaf' shaped zone of inhibition by the test strain was considered positive for Carbapenemase production and the negative strain showed an undistorted zone of inhibition (Figure 1). [9]

Double Disk Synergy Test: A lawn culture of overnight suspension of the test strain adjusted to 0.5 McFarland turbidity standard was inoculated onto Mueller-Hilton agar. 0.5 M EDTA solution was prepared by dissolving 186.1g of disodium EDTA in 1000ml of distilled water and p^H adjusted to 8.0 using NAOH. A 10 µg Imipenem disk and EDTA disk [10 µl of 0.5M EDTA] was placed 10 mm apart from edge to edge. After incubating overnight at 37°C, the presence of an expanded growth inhibition zone between the two disks was interpreted as positive for MBL production and the negative strain showed no synergistic zone of inhibition (Figure 2). [9]

Disk Potentiation Test: A lawn culture of the 0.5 McFarland turbidity matched test strain was inoculated onto Mueller-Hilton agar. Two 10 µg Imipenem disks were placed wide apart with 5 µl of 0.5M EDTA solution added to one Imipenem disk. After overnight incubation, an increase in zone size≥7mm of around the Imipenem EDTA disk as compared to Imipenem disk only was considered as positive for MBL production (Figure 2). [10]

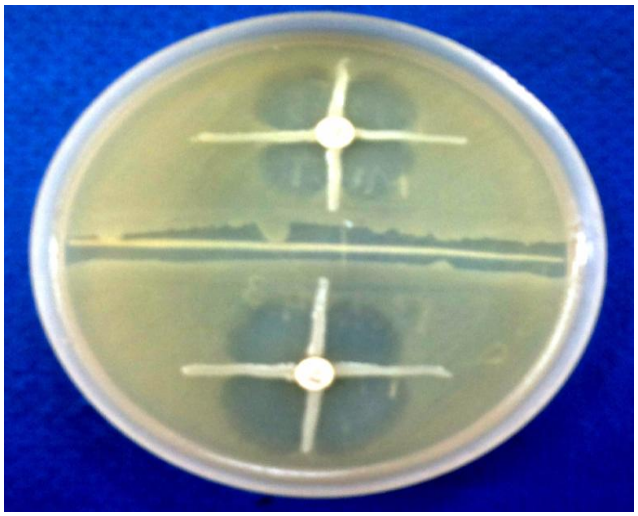

Figure 1: Modified Hodge test with test strain (above) and control strain (below)

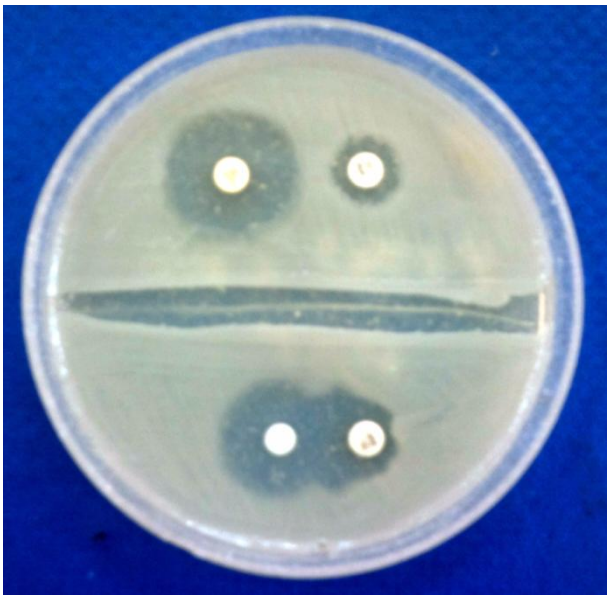

Figure 2: Disk Potentiation test (above) and Double disk Synergy test (below)

III. Results

A total of 2548 clinical samples were collected during the study period from June 2011 to August 2011 from various wards. 1680 samples [65.9%] were collected from male patients and 868 [34.1%] were collected from female patients. The ward wise distribution of the clinical samples is given in Table 1.

Table 1: Ward wise distribution of the clinical samples:

Sl No.	Ward	Samples
1	Medicine ward	645
2	Surgery ward	753
3	Orthopedic ward	702
4	Pediatric ward	198
5	ICU	250
	Total samples	2548

Of the total 2548 clinical samples, 850 were urine samples, 845 were blood culture samples, 547 were pus samples, 243 were sputum samples, 38 were throat swabs and 25 were endotracheal tube suction samples. Among 2548 clinical samples, 98 [3.85%] isolates of Pseudomonas and 69 [2.71%] isolates of Acinetobacter were isolated. Table 2 shows the isolation of Pseudomonas and Acinetobacter species from various clinical samples.

Table 2: Pseudomonas and Acinetobacter species from various clinical samples.

Sl No.	Clinical samples	No. of samples	Pseudomonas		Acinetobacter	
			No.	%	No.	%
1	Urine	850	2	0.24	3	0.35
2	Blood	845	5	0.59	18	2.13
3	Pus	547	64	11.7	27	4.94
4	Sputum	243	17	6.99	12	4.94
5	Throat swab	38	1	2.63	1	2.63

6	Endotracheal tube suction	25	9	36.0	8	32.0
	Total samples	2548	98	3.85	69	2.71

A very high percentage of Pseudomonas [36%] and Acinetobacter [32%] were isolated from Endotracheal tube suction samples followed by pus samples and very low percentage of Pseudomonas [0.24%] and Acinetobacter [0.35%] isolated from urine samples. Among 98 Pseudomonas isolates, 15 [15.3%] strains were resistant to Imipenem and among 69 Acinetobacter isolates, 30 [43.5%] strains were found resistant to Imipenem. Higher rate of Imipenem resistance is seen among acinetobacter species than among pseudomonas species. Table 3 shows sensitivity pattern of Pseudomonas and Acinetobacter species to Imipenem.

Table 3: Sensitivity pattern of Pseudomonas and Acinetobacter to Imipenem

Sl No.	Clinical samples	Pseudomonas		Acinetobacter	
		Sensitive	Resistant	Sensitive	Resistant
1	Urine	2	-	1	2
2	Blood	5	-	14	4
3	Pus	52	12	10	17
4	Sputum	16	1	9	3
5	Throat swab	1	-	-	1
6	Endotracheal tube suction	7	2	5	3
	Total	83	15	39	30

All pseudomonas strains isolated from urine, blood and throat swab were sensitive to Imipenem. 17 [63%] acinetobacter isolates from pus samples were resistant to Imipenem while only 10 [37%] isolates were sensitive to Imipenem. 15 Pseudomonas and 30 acinetobacter strains, which were resistant to imipenem by disc diffusion method were screened for MBL detection by Modified Hodge test, Double disk synergy test and Disk potentiation test. The results of the three tests are given in table 4.

Table 4: Results of Modified Hodge test, Double disk synergy test and Disk potentiation test:

Sl No.	Organism	No. of Imipenem resistant isolates	No. of Positives (%)		
			Modified Hodge test	Double disk synergy test	Disk potentiation test
1	Pseudomonas	15	7(46.7)	1(6.7)	1(6.7)
2	Acinetobacter	30	21(70)	2(6.7)	2(6.7)

Among 15 Imipenem resistant Pseudomonas isolates, 7 [46.7%] isolates were positive for carbapenemases by Modified Hodge test, while only one [6.7%] strain was positive for metallo-β-lactamase production by both Double disk synergy test and Disk potentiation test. Among 30 Imipenem resistant Acinetobacter isolates, 21 [70%] isolates were positive for carbapenemases by Modified Hodge test, while two [6.7%] strains were positive for metallo-β-lactamase production by both Double disk synergy test and Disk potentiation test. The sensitivity pattern of Pseudomonas and Acinetobacter to other antibiotics is given in Table 5. All strains of MBL producing Pseudomonas and Acinetobacter species were found to be multidrug resistant.

Table 5: Sensitivity pattern of the Pseudomonas and Acinetobacter strains

Drugs	Pseudomonas				Acinetobacter			
	MBL Positive		MBL Negative		MBL Positive		MBL Negative	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Imipenem	0	1	83	14	0	2	39	28
Ceftazidime	0	1	33	64	0	2	15	52
Chloramphenicol	0	1	12	85	0	2	7	60
Ciprofloxacin	0	1	75	22	0	2	44	23
Amikacin	0	1	63	34	0	2	44	23
Piperacillin& tazobactam	0	1	73	24	0	2	41	26

IV. Discussion

Pseudomonas and Acinetobacter species are mainly associated with multidrug resistant nosocomial infections. One of the commonest causes for multidrug resistance among these species is the production of Carbapenemase. Carbapenemases are beta lactamases which have the ability to hydrolyze penicillins, cephalosporins, carbapenems and monobactams. Production of MBL by Pseudomonas and Acinetobacter has tremendous therapeutic consequences, since these organisms also carry multi drug resistance genes and the only treatment option is Polymyxin, Colistin and tigecycline.

Of the 45 samples which yielded Imipenem resistant isolates, 37 (82%) were collected from patients who have had significant hospital exposure i.e., more than 72 hours of hospital stay. Also, 22(48%) patients among these have had interventional procedures such as surgery, tracheostomy, intubation, catheterization of the bladder, central IV line etc.

In our study, we report 15.3% Imipenem resistance among Pseudomonas strains. However a higher resistance rate have been reported by Varaiya et al (25%) [11], Noyal et al (31.1%) [12] and by Andrade et al (40%) [13]. A lower resistance rate of 10.9% have been reported by Kanungo et al. [14] Imipenem resistance among Acinetobacter in 43.5% in our study. Similarly high rate of Carbapenem resistance have also been reported by Noyal et al (59%) [12] and Peleg et al (64%) [15] . Sinha et al have reported a very low rate of 14%. [16] Indiscriminate and irrational use of antibiotics have led to increase in the incidence of Carbapenem resistance among Pseudomonas and Acinetobacter species.

In our study, 46.7% of Imipenem resistant Pseudomonas isolates were positive for carbapenemase production, but only 6.7% of them showed Metallo-β-lactamase production. These strains were mainly isolated from pus and endotracheal tube suction samples. Similar to our study, lower rate of MBL producing Pseudomonas have been reported by Agrawal et al (8.05%), [17] Mendiratta et al (8.2%), [18] Attal et al (11.4%), [19] Navneeth et al (12%) [20] and Hemlata et al (14%) [21]. A very high rate of MBL production among Pseudomonas has been reported by Noyal et al (50%) [12] and Jesudasan et al (75%) [22].

Eventhough 70% of our Imipenem resistant Acinetobacter species were Carbapenemase producers, only 6.7% of them were MBL producers, which is similar to the study reported by Noyal et al (6.5%) [12]. In the study by Sinha et al [16], none of the Meropenem resistant Acinetobacter isolates were MBL producers. But our study reports a very high rate (70%) of carbapenemase production among Acinetobacter species, which is alarming, as these isolates are also resistant to Cephalosporins, Aminoglycosides and Quinolones, making the treatment option very narrow with only Colistin or Tigecycline.

Six isolates of Imipenem resistant Pseudomonas and 19 isolates of Imipenem resistant Acinetobacter were positive for carbapenemase by Modified Hodge test, but were found to be negative for MBL. This may be because of the production of carbapenemase other than MBL, which is not dependent on zinc ion for its action, ie., serine based carbapenemases. Apart from class B metallo betalactamases, Class A and D Carbapenemases, which have serine based hydrolytic mechanism, are responsible for imipenem resistance among Pseudomonas and Acinetobacter species. The other reason for carbapenem resistance other than MBL production is due to impermeability, which occurs due to loss of the opr D porin and due to the upregulation of an effective efflux system present in these organisms. [23] So, detection of both MBL and carbapenemase is necessary in deciding the treatment option.

V. Conclusion

With the increasing use of carbapenems for treating infections with ESBL producing organisms, the problem of MBL production is also increasing. Eventhough MBL production is an important mechanism of Carbapenem resistance among Pseudomonas and Acinetobacter species, other mechanisms are also seen at a higher rate. Modified Hodge test can be used as a screening test for the detection of carbapenemases including MBL production. Both double disk synergy test and disk potentiating tests are equally effective in the detection of MBL. These phenotypic tests are easy to perform and cost effective and can be introduced for routine detection of MBL in the laboratory.

References

[1]. Watanabe S, Iyobe S, Inoue M, Mitsuhashi S. Transferable imepenem resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother. 1991; 35:147-51.

[2]. Navon-Venezia, S., Ben-Ami, R. & Carmeli, Y. Update on Pseudomonas aeruginosa and Acinetobacter baumannii infections in the healthcare setting. Current Opinion Infectious Diseases. 2005. 18. 306–313.

[3]. Govan JRW, Pseudomonas, Stenotrophomonas, Burkholderia,In: College JG, Fraser AC, Marmion BP, Simmons A, editors Mackie & McCartney practical medical microbiology. 14th ed. New York, London. Churchill livingstone; 1996. 413-24.

[4]. R. Uma Karthika, R. Srinivasa Rao, Suchismita Sahoo, P. Shashikala, Reba Kanungo, S. Jayachandran and K. Prashanth. Phenotypic and genotypic assays for detecting the prevalence of metallo-b-lactamases in clinical isolates of Acinetobacter baumannii from a South Indian tertiary care hospital. Journal of Medical Microbiology. 2009. 58. 430–435

[5]. Bush, K. New β-lactamases in gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. Clin. Infect. Dis. 2001. 32.1085– 1089

[6]. Fluit, A. C., and F. T. Schmitz. Class 1 integrons, gene cassettes, mobility, and epidemiology. Eur. J. Clin. Microbiol. Infect. Dis. 1999. 18. 761– 770.

[7]. Gian Maria Rossolini, Elisabetta Mantengoli, Jean-Denis Docquier, Rosa Anna Musmanno, Grazietta Coratza. Epidemiology of infections caused by multiresistant Gram-negatives: ESBLs, MBLs, panresistant strains. New Microbiologica. 2007. 30. 332-339

[8]. Clinical Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests , Approved standard, 9th ed. CLSI documentM2 A9.CLSI,Wayne,PA; 2006.

[9]. Lee K, Chong Y, Shin H B, Kim YA, Yong D, Yum JH. Modified Hodge and EDTA disk synergy tests to screen metallo β lactamase producing strains of Pseudomonas and Acinetobacter species. Clin Microbiol Infect. 2001;7:88-91

[10]. Yong D, Lee K, Yum JH, Shin HB, Rossolinism, Chong Y. Imipenem – EDTA disk method for differentiation of metallobetalactamases producing clinical isolates of Pseudomonas spp and Acinetobacter spp. J Clin Microbiol. 2002;40:3798-801

[11]. Ami Varaiya, Nikhil Kulkarni, Mana si Kulkarni, Pallavi Bhalekar & Jyotsana Dogra. Incidence of metallo beta lactamase producing Pseudomonas aeruginosa in ICU patients. Indian J Med Res. 2008;127:398-402

[12]. M.J.C. Noyal, G.A. Menezes, B.N. Harish, S, Sujatha & S.C.Parija. Simple screening tests for detection of carbapenemases in clinical isolates of nonfermentative Gram negativebacteria. IndianJMed Res. 2009. 129. 707 712.

[13]. Andrade SS, Jones RN, Gales AC, Sader HS. Increasing prevalence of antimicrobialresistanceamong Pseudomonas aeruginosa isolatesinLatin American medical centres: 5 year report of the SENTRY Antimicrobial SurveillanceProgram 1997–2001. J Antimicrob Ch emother. (2003). 52. 140- 1.

[14]. KanungoR,SrinivasanS,DeviS.Emergingresistanceto carbapenemsin hospital acquired Pseudomonas infection: a cause for concern. Indian J Pharmacol. 2006. 38. 287- 8.

[15]. Peleg AY, Franklin C, Bell JM, Spelman DW. Emergence of carbapenem resistance in Acinetobacter baumannii recovered from blood cultures in Australia. Infect Control Hosp Epidemiol. 2006. 27. 759-61.

[16]. Sinha M, Srinivasa H. Mechanisms of resistance to carbapenems in meropenem resistant Acinetobacter isolates fromclinicalsamples. Indian J MedMicrobiol. 2007. 25. 21- 5.

[17]. AgrawalG,LodhiRB,KamalakarUP,Khad seRK, JalgaokarSV.Studyof metallobetalactamases production in clinical isolates of P. aeruginosa. IndianJMedMicrobiol . 2008. 26. 349 – 51

[18]. MendirattaDK,DeotaleV,NarangP.Metallobetalactamasesproducingof P.aeruginosainhospitalfromruralarea. IndianJMedRes . 2005 121. 701 – 3.

[19]. Attal Ro , Basak S, Mallick S K, Bose S . Metallobetalactamase producing Pseudomonas aeruginosa: An emerging threat to Clinicians. J of Clinical and DiagnosticResearch. 2010. 4. 2691-96.

[20]. NavaneethBV,SridaranD,SahayD,BelwadiMRS. Apreliminarystudyon metallo β lactamase producing Pseudomonas aeruginosa in hospitalized patients. IndianJ MedRes . 2002. 116. 264 7.

[21]. Hemalatha V, Sekar U, Kamat V. Detection of metallobetalactamases producing P.aeruginosa in hospitalized patients. Indian J Med Res .2005. 122.148 – 52.

[22]. Jesudason MV, Kandathil AJ, Balaji V. Comparison of two methods to detect carbapenemase & metallo β lactamase production in clinical isolates. Indian J Med Res. 2005. 121. 780-3

[23]. Nordmann P. Poirel L. Emerging carbapenemases in Gram negative aerobes. Clin Microbial.Infect. 2002; 8. 321 31