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Original Research Paper

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DETECTION OF METALLO-BETA-LACTAMASE PRODUCING PSEUDOMONAS AND ACINETOBACTER SPECIES FROM DIFFERENT CLINICAL SPECIMENS IN A TERTIARY CARE HOSPITAL AT PUDUCHERRY.

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ABSTRACT **Introduction** Pseudomonas and Acinetobacter species are frequently isolated non-fermenting gram negative bacteria in a variety of hospital acquired infections. Metallo-beta-lactamases have become a serious threat in treating infections because of their multiple drug resistance including carbapenems. **Objectives** To determine the prevalence of MBL production among Pseudomonas and Acinetobacter species and to evaluate the different phenotypic MBL detection methods. **Materials and methods** A total of 104 isolates of carbapenem resistant Pseudomonas (78) and Acinetobacter (26) from different clinical specimens were tested for MBL production by Modified Hodge Test, Combined Disk Test and Double Disk Synergy Test. Antibiotic susceptibility was performed by Kirby- Bauer Disk Diffusion method. **Results** Pseudomonas aeruginosa (11.29%) Acinetobacterbaumanii (11.53%) were the predominant MBL producers. MBL production was detected 61.53%, 84.61% and 38.46 % by DDST(Doule disc synergy test), CDT (Combined disc test), and MHT (Modified Hodge test) respectively. Colistin and Polymyxin B are the only option available for treating such infections. **Conclusion** MBL production among Pseudomonas and Acinetobacter species are increasing due to the continuous use of carbapenems and selective antibiotic pressure. Strict antibiotic policy and infection control practices help prevent the further spread.

KEYWORDS : Metallo beta lactamases, Pseudomonas, Acinetobacter

INTRODUCTION

One of the most important mechanism of antimicrobial resistance by bacteria to betalactam group of antibiotics are due to the production of various betalactamases like extented spectrum betalactamases (ESBL), AmpCbetalactamases, Carbapenemases, etc. Carbapenems are often used as a last resort against betalactamases producing bacteria due to their stability against ESBLs and AmpCbetalactamases. However there has been an increase in resistance to carbapenems especially by Psuedomonas and Acinetobacter species due to production of carbapenemases like Metallo beta lactamases (MBL). MBLs are melalloenzymes of Ambler class B and are resistant to clavulanic acid, sulbactam, tazobactam, etc. They have zinc in their active site and are inhibited by ethylene-diamine tetra acetic acid (EDTA).[1]The rapid dissemination of MBL producing isolates in hospital require an effective phenotypic method for detecting them where there is limited resource. This study aims to provide an early, effective and user friendly phenotypic method for identifying MBLs among Pseudomonas and Acinetobacter species isolated from various clinical samples.

MATERIALS AND METHODS

A total of 104 non duplicate isolates of Pseudomonas (78) and Acinetobacter species (26) were isolated from various clinical samples of pus, urine, sputum, blood, bronchial secretions, endotracheal aspirates, etc. over a period of one year. Antibiotic susceptibility was done by Kirby-Bauer disc diffusion method as recommended by CLSI guidelines. Amikacin (30μg), Gentamicin (10μg), Ceftazidime (30μg), ceftriaxone (30μm), Cefepime (30μg),Ciprofloxacin (5μg), Piperacillin (100μg), Piperacillin-tazobactam(100μg+10μg), Imipenem(10μg), Meropenem(10μg) and Colistin (50μg) were the antibiotics tested.

MIC of Imepenem by Agar dilutuion method

MIC was determined on Mueller Hinton agar plate with serial double dilutions for imipenem ranging from 0.125μg/ml to 256μg/ml concentrations. ATCC Pseudomonas aeruginosa 27853 was used as control strain. MIC of 8μg/ml or above was interpreted as resistant to imipenem.[2]

Test for metallo beta-latamase detection

Modified Hodge test

A 0.5Mc Farland suspension of ATCC E.coli 25922 was lawn cultured on MHA plate. An imipenem 10μm disc was placed at the center of the plate. Test strain was streaked from edge of the disc to periphery of the plate in four different directions. After overnight incubation at 37°C, presence of a clover leaf shaped zone of inhibition was considered as positive.[3]

Imipenem-EDTA double disc synergy test

A 0.5 McFarland suspension of test organism was lawn cultured on MHA plate. An imipenem disc (10μm) was placed 20mm center to center from a blank disc (6mm diameter whatmann filter paper no. 1) containing 10μl of 0.5 m EDTA (750μg). After overnight incubation at 37°C, presence of an expanded zone of inhibition towards the EDTA disc was interpreted positive for MBL production.[3]

Imipenem-EDTA combined disc test

A 0.5 McFarland suspension of test organism was lawn cultured on MHA plate. Two imipenem disc (10μm) were placed 20 mm apart and 10μl of 0.5 m EDTA (750μg) was added to one of them. After overnight incubation at 37°C, increase in inhibition zone with imipenem-EDTA disk of $\geq$ 7mm than imipenem disc alone was interpreted positive for MBL production.[4]

RESULTS

A total of 104 isolates (Pseudomonas spp. 78 and Acinetobacter spp. 26) were cultured from different clinical samples. Maximum isolates were obtained from pus (60.57%) followed by tracheal aspirate (15.38%) and sputum (6.73%). 29 (27.88%) were from surgical wards followed by 17 from ICUs (16.35%) and 13 from medical wards(12.5%). Risk factors

associated with these infections were post-surgical (23%), prolonged ICU stay (16.34%) and catheter related (15.38%). Carbapenem resistance was seen in 12.5% (13/104) of the total isolates (Pseudomonas spp. (9/78), Acinetobacter spp. (4/26)). 69.33% of them showed high level resistance MIC > 32μg/ml. MBL production was detected in 5/13 (38.46%), 8/13(61.53%), 11/13 (84.61%) by MHT, DDST, CDT respectively. MBL was detected in 12/13 (92.30%) among the carbapenem resistant isolates by different phenotypic methods. One of the isolate could not be detected by any of these methods.

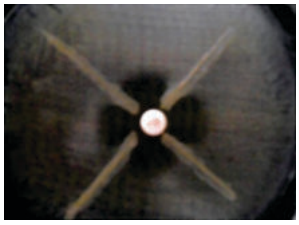

Figure 1: MHT positive

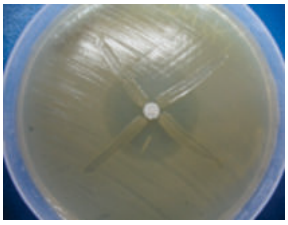

Figure 2: MHT negative

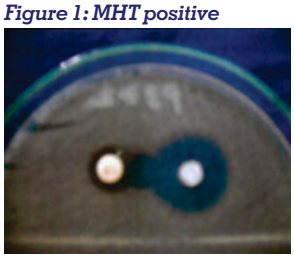

Figure 4: DDST positive

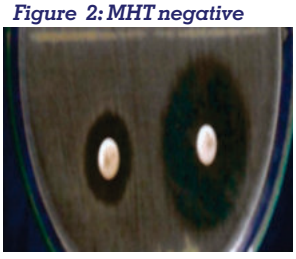

Figure 3: DDST negative

Figure 5: CDT positive

Figure 6: CDT negative

Table 1: Comparison of MBL detection by different phenotypic methods.

Organism	MBL detected	MHT		DDST		CDT	
		Positive	Negative	Positive	Negative	Positive	Negative
Pseudomonas spp.	8	3	6	5	4	8	2
Acinetobacter spp.	4	2	2	3	1	3	1
Total	12	5	8	8	5	11	3

DISCUSSION

Pseudomonas and Acinetobacterspp were predominantly cultured from pus samples (60.57%) followed by tracheal aspirate (15.38%) and sputum (6.73%). Kharangate et al has reported 50% incidence in pus samples. Gladstone et al from CMC vellore showed a higher incidence in endotracheal aspirate (42.4%).[5,6]

Majority of samples were from surgical wards (27.88%). Most of the studies from India shows higher prevalence from surgical wards, may be due to prolonged stay in hospital following surgeries. The second highest source was in ICUs (16.34%) that clearly indicate the importance of infections caused by Pseudomanas andAcinetobacter spp. in intensive care settings. Several studies also report a higher incidence in these units.

The significant risk factor in the present study were surgery (23%), ICU stay (18.26%) and catheter associated (15.38%). Studies by Joshy et al (30.8%) and Ezeltahawy et al (34%) identified longer stay in ICUs as risk factor.[7,8]Present study also observed invasive procedures like intubation, catheter lines, dialysis and prolonged therapy with antibiotics as major risk factors.

Carbapenem resistance was observed in 12.5% isolates (13/104) is comparatively low with most of the Indian studies but comparable to the reports by Berger et al (9.2%), Prakash et al (11.3%), Kanugo et al (10%) and Glad stone et al (12.2%). All the carbapenem resistant isolates were susceptible to Colistin. High level resistance MIC >32μg/ml was observed in 9 isolates (69.23%) are comparable to the studies by Jones et al and Masaru et al.[9,10]

Among thecarbapenem resistant isolates MBL was detected in 12/13 (92.30%) by different phenotypic methods. MBL production was observed in tracheal aspirates (16.66%) followed by pus (14.28%) comparable to the study by Gian Mania et al 13% and 10% respectively.[11]Among the different phenotypic methods CDT detected 11/13 (84.61%), DDST detected 8/13 (61.53%) and MHT detected 5/13 (38.46) MBL producers.

The present study showed CDT as simple, inexpensive and user friendly phenotypic screening test for MBL production among Pseudomonas and Acinetobacter spp. Several studies from Ting tingQu et al, Dongeun Yong et al, Pitout et al, Behera et al, Lee et al, Berges el al also shows CDT as better method of choice to detect MBL production in the absence of PCR.[12,13.]

REFERENCES:

1. Jonaidijafari N, RanjbarR,et al 2009 The study of prevalence and antimicrobial susceptibility of tracheal bacterial strains isolated from pediatric patients *Pak J Biol Sci*.;12(5):455-8
2. Vijaya D, K.Bhavani S, Veena M,2000 Prevalence of nonfermenters in clinical specimens:54:87-91*Indian journal of medical sciences* volume54, issue3p8791.
3. Lee K, Lim YS, Yong D, et al 2003 Evaluation of Hodge test and Imepenen-EDTA double-disk Synergy test for differentiating metallo-β-lactamase producing isolates of *Pseudomonas spp. and Acinetobacterspp. JClinMicrobiol 41 : 4623-9*
4. YooChul Lee, Byung Jun, et al 2007 Molecular characterization of *Pseudomonas aeuroginosa* isolates resistant to all antimicrobial agents, but susceptible to Colistin*The Journal of Microbiology* p 358-363
5. Kharangate NV, Pinto MJ, Rodrigues S.et al 2001 Characterization of nonfermenters from clinical samples *J Assoc Physicians India*.;49:324-6
6. Gladstone P, Rajendran P,Rahmanadathan KN 2005, Incidence of carbapenem resistant nonfermenting gram negative bacilli from patients with respiratory infections in th intensive care units. *Indian J MdMicrobiol*23: 189-91
7. Joshi SG, Litake GM, Satpute MG. et al 2006 Clinical and demographic features of infection caused by *Acinetobacter*species. *Indian J MedSci*; 60:35160
8. Ezeltahawy A.T.A.E., Khalaf R.M.F. 2001 Antibiotic Resistance among Gram negative nonfermentative bacteria at a teaching hospital in Saudi Arabia *Journal of Chemotherapy*vol 13 p 260-4
9. Jones RN, Pfaller MA, Marshall SA,. Et al 1997 Antimicrobial activity of 12 broad spectrum agents tested against 270 nosocomial blood stream infection isolates cused by non-enteric gram negative bacilli: occurrence of resistance, molecular epidemiology and screening for metallo-enzymes. *DiagnMicrobiol Infect Dis*. 29 (3):187 – 92
10. Masaru Ohara, Shuntaro Kouda, Makato Onodera, 2007, Molecular characterization of Imepenam resistant Psuedomonasaerugenosa in Hiroshima Japan. *MicrobiolImmunol*, 51 (3),271-77
11. Gian Maria Rossolini, Francesco Luzzaro, Roberta Migliavacca, et al 2008 First country wide survey of acquired Metallo-β-lactamases in Gram negative pathogens in Italy *Antimicrobial agents and Chemotherrapy*, p.4023-4029 vol.52,no. 11
12. Lee K, Lim YS, Yong D,.et al 2003 Evaluation of the Hodge test and the imipenem-EDTA double disk synergy test for differentiating metallo-β-lactamase producing isolates of *Pseudomonas spp. and Acinetobacter spp. J ClinMicrobiol*41 : 4623 – 9
13. Berges L, Rodriguez-Villalobos, Deplano et al 2007 Prospectivee evaluation of imepenem / EDTA combined disk and E test for detection of metallo-β-lactamase producing *Pseudomonas aeruginosa Journal Antimicribial Chemotherapy*. 59(4):812 - 813