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DETECTION OF METALLO BETA LACTAMASE (MBL) PRODUCING PSEUDOMONAS AERUGINOSA IN BURNS WOUND INFECTION

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Abstract : BACKGROUND Prevalence of Pseudomonas aeruginosa infection is common among burns patients. Metallo-beta-lactamases (MBLs) produced by clinical isolates of Pseudomonas aeruginosa has increased considerably in recent years. This may cause phenotypic resistance to virtually all clinically available bate lactams. The drug resistance due to MBL has a potential for rapid spread to other microorganisms AIMS AND OBJECTIVES To detect the MBL producing Pseudomonas aeruginosa from burns wound infections and to study their antibiotic sensitivity pattern METHOD Pseudomonas aeruginosa were isolated from infected burns wounds samples. Imipenem resistant isolates were further tested by double disc synergy test (DDST) and Combined Disc Diffusion Test (CDDT).RESULTS Out of total 156 Pseudomonas aeruginosa isolates, 58 (37.1) were imipenem resistant, among which, 42(26.92) isolates were MBL producers by double disc synergy test (DDST) and 46 (29.48) were MBL producers by Combined Disc Diffusion Test (CDDT). MBL producers were resistant to commonly used antibiotics than non MBL producers. All isolates were sensitive to colistin (10micrograms) and polymyxin B (300 units). CONCLUSION MBL production is an important mechanism of carbapenem resistance among Pseudomonas aeruginosa . Double disc synergy test (DDST) and Imipenem- EDTA Combined Disc Diffusion Test (CDDT) can be used for detection of MBL Production. So screening method should be done routinely to determine changing trends in the type of organisms and their resistance pattern. Patients infected with MBL producing organisms should be promptly treated. Antibiotic stewardship programme should be made which is the main stay in the management of burns wound infection and to prevent the spreading of antibiotic resistance.

Keyword : Pseudomonas aeruginosa , MBL, Imipenem, CDDT, DDST

INTRODUCTION:

Infections leading to septicemia are the common cause of morbidity and mortality following burns injury , although burns wound surfaces are sterile immediately following thermal injury, these wounds usually become infected with microorganisms. Hospital acquired infections in burns patients can be endogenous (patients own flora) or exogenous (hospital environment and personnel). The worldwide emergence of antimicrobial resistance among burns wound pathogens, Particularly nosocomial isolates, limits the available antibiotics for effective treatment of burns wound infections. A well planned surveillance and infection control practices including preventive measures can help to reduce the incidence of hospital acquired infections amongst burns patients. 1 *Pseudomonas aeruginosa* is one of the most common pathogens causing burns wound infections. Both intrinsic and acquired drug resistance mechanisms are frequent in nosocomial isolates of *Pseudomonas aeruginosa* and often involves more than one antimicrobial class.2 Acquired metallo- lactamases (MBL) have recently emerged as one of the common resistance mechanisms owing to their capacity to hydrolyze all -lactams including carbapenems and also because their genes are carried on plasmids, the highly mobile elements, allowing easy dissemination. Such strains are not susceptible to therapeutic serine lacatamase inhibitors such as tazobactam, clavulanic acid & sulbactams.3 In recent years, MBL genes have spread from *Pseudomonas aeruginosa* to members of Enterobacteriaceae by Horizontal gene transfer, could simulate the global spread of resistance pattern.2,3 MBL producing isolates are also associated with a higher morbidity and mortality.

The occurrence 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. Genotypic methods of detection of MBL producing organisms give specific and accurate results, but its use is limited by the cost and infrastructural as well as technical requirements, especially in a health care institute serving rural population.10,13,14. So Several non molecular techniques have been studied, all taking advantage of the enzymes zinc dependence by using chelating agents, such as EDTA or 2 mercaptopropionic acid, to inhibit its activity. Carbapenems and cephalosporin/inhibitor combinations are being used in these infections since the last few years. Therefore, we envisaged to ascertain the occurence of MBL producing *Pseudomonas aeruginosa* in our hospital .

The present study was thus planned to evaluate the pattern of MBL producing *Pseudomonas aeruginosa* from wound swabs from patients with burns injury, to determine the antimicrobial susceptibility pattern of the isolates by Kirby Bauer disc diffusion method as per CLSI guidelines3. Continuous monitoring of microbial spectrum and their

antibiogram is absolutely essential to determine changing trends in resistance pattern, thus helping in judicious use of antibiotics by framing an appropriate antibiotic policy, which is the mainstay in management of patients with burns wound infections.

Aims And Objective:

1. To isolate and identify *Pseudomonas aeruginosa* from burns wound infection.
2. To detect the Antibiotic susceptibility pattern of the isolated *Pseudomonas aeruginosa* .
3. To detect Metallo beta lactamase (MBL) producing *Pseudomonas aeruginosa* by Double disc synergy test (DDST) and Combined Disc Diffusion Test (CDDT).

Materials And Methods:

This Cross sectional study was conducted in a tertiary care hospital for a period of 6 months with an inclusion criteria of all *Pseudomonas aeruginosa* isolated in pure cultures from burns wound infection and the exclusion criteria of all gram positive and other gram negative bacterias isolated from burns wound infection. A total of 156 *Pseudomonas aeruginosa* were isolated as pure culture from burns wound infection. *Pseudomonas aeruginosa* were identified based on colony morphology, Gram staining and biochemical reactions4. Antibiotic sensitivity test was done by Kirby- Bauer disc diffusion method as per CLSI 2013 guidelines. Sensitivity pattern for the following antibiotics were determined. Amikacin(30µg), Gentamicin (10µg), Tobramycin(10µg), Ceftazidime(30µg), Cefepime(30µg), Cefoperazone sulbactum (75/10µg), Piperacillin / Tazobactam (100/10µg), Ciprofloxacin (5µg), and Imipenem(10µg), Meropenem(10 µg), Polymyxin B(300 units), Colistin(10µg). MBL producers were detected by Phenotypic methods to all imipenem resistant isolates of *Pseudomonas aeruginosa* .

Imipenem-EDTA double disc synergy test (DDST):

The IMP-EDTA double disc synergy test (DDST) was performed by inoculating the test organisms in a Mueller Hinton agar as recommended by the CLSI guidelines.[5] An Imipenem (10 g) disc was placed 20 mm centre to centre from a blank disc, 10 L of 0.5 M EDTA solution (750 g) was added to the blank disc. Plates were incubated at 37°C over night. 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 MBL positive. 7,8,9

Imipenem (IMP)-EDTA combined disc diffusion test: [CDDT]

The IMP-EDTA combined disc test (CDDT) was performed by inoculating test organisms in a Mueller Hinton agar as recommended by the CLSI guidelines.5 Two 10 g imipenem discs were placed on lawn culture which was inoculated with 0.5 Mc Farland standard broth culture and 10 L of 0.5 M EDTA solution (750 g) was added to one of the disc to obtain the desired concentration. The inhibition zones of the imipenem and imipenem-EDTA discs were compared after 16 to 18 hours of incubation at 35°C. In the combined disc test, if the increase in inhibition zone with the Imipenem and EDTA disc was 7 mm than the Imipenem disc alone, it was considered as MBL positive .1,3,6

RESULTS:

Table no:1 Imipenem Sensitivity of *Pseudomonas aeruginosa* strains (n=156)

Total no of isolates	IMP Sensitive	IMP resistant
156	98 (62.8%)	58 (37.2%)

Table no:2 Detection of MBL producing *Pseudomonas aeruginosa* by double disc synergy test (DDST) (n=156)

MBL positive	MBL negative 114 (73.05%)	
	Imipenem Resistant	Imipenem Sensitive
42(26.92%)	16(10.25%)	98(62.8%)

Double Disc Synergy Test (DDST)

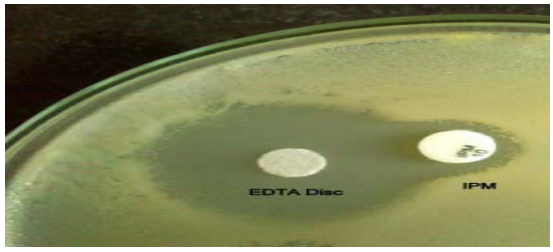

Table no: 3 Detection of MBL producing *Pseudomonas aeruginosa* by Combined Disc Diffusion Test (n=156)

MBL positive	MBL negative 110 (70.51%)	
	Imipenem Resistant	Imipenem Sensitive
46 (29.48%)	12 (7.69%)	98 (62.80%)

Combined Disc Diffusion Test (CDDT)

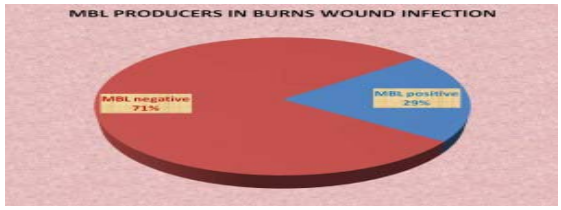

Table no:4 Antimicrobial sensitivity of MBL positive and MBL negative isolates in burns wound infection

Antibiotics (µg)	Sensitivity of MBL Producers (n=46)	Sensitivity of MBL non producers (n=110)
Amikacin(30)	2(4.34%)	74(67.27%)
Gentamicin(10)	0	28 (25.45%)
Tobramycin (10)	0	26(23.64%)
Ceftazidime (30)	0	110(100%)
Cefepime(30)	0	84(76.36%)
Cefoperazone sulbactum(75/10)	9 (19.6%)	71(64.55%)
Piperacillin/tazobactam(100/10)	16 (34.70%)	110(100%)
Ciprofloxacin (5)	3 (6.52%)	62(56.36%)
Imipenam (10)	0	110(100%)
Meropenam (10)	4(8.69%)	110(100%)
Polymyxin B(300)	46(100%)	110(100%)
Colistin (10)	46(100%)	110(100%)

DISCUSSION:

Pseudomonas aeruginosa is one of the important organism responsible for outbreak of hospital acquired infection worldwide. High rate of infection in burns patients is due to loss of protective barrier of skin and presence of devitalized tissues supporting the growth of microorganisms and biofilm production. Biofilm produced by *Pseudomonas aeruginosa* inhibits penetration of systemically administered antibiotics. MBL producing organisms are more resistant to commonly used antibiotics and carbapenems.13Resistance to carbapenems is due to increased efflux system, decreased outer membrane permeability, alteration of penicillin binding proteins and production of carbapenem hydrolyzing enzymes

3,8,13. The first MBL, encoded on a plasmid, IMP–1(“active on imipenem”), was discovered in Japan in 1988. In India, MBL producing *Pseudomonas aeruginosa* was first reported in 2002.8,10,13 According to the Ambler scheme of molecular classification of carbapenemase, MBL falls into class B category. MBLs belong to IMP, VIM (for “Verona integron- encoded Metallo- -lactamases”), GIM (for “German imipenemase”) and SIM (for “Seoul imipenemase”) families. SPM–1(for “SaoPaulo Metallo-lactamases”), was a new family isolated in *Pseudomonas aeruginosa*. According to Ami Varaiya6 varying resistance of 4-60% to imipenem has been reported world wide. But in this study , Out of the 156 isolates 98 (62.8%) isolates were Imipenem sensitive and 58 (37.1%) isolates were imipenem resistant by disc diffusion method ,this was comparable with Purohit study 12 (31.54%),Ami varaiya6(25%),But Buchunde 2 showed only 17.8% resistance and Hemalatha 11 showed 16% resistance. Among 58 (37.1%) Imipenem resistant isolates 42 (26.9%) isolates were MBL producers by Double disc synergy test (DDST) and 46(29.48%) isolates were MBL producers by Combined disc diffussion test (CDDT). The remaining 12 (7.6%) of the Imipenem resistant *Pseudomonas aeruginosa* were not detected by Combined disc diffussion test (CDDT) may be because of the production of carbapenemase other than MBL, which is not dependent on Zinc ion for its action. Apart from MBL, other classes of carbapenemases class A or D can also be responsible for Imipenem resistance in *Pseudomonas aeruginosa* .10 Various studies have reported the uses of different methods .2,3,12 In this study we have employed Double disc synergy test (DDST) and Combined disc diffussion test (CDDT) methods to detect MBL producers. As this is not a comparative study and Genotypic methods were not performed, Double disc synergy test (DDST) and Combined disc diffussion test (CDDT) could not be compared with a known standard to calculate sensitivity, specificity, positive and negative predictive value.

The observation of zone enhancement made in Double disc synergy test (DDST) may have subjective variation in interpretation.But in Combined disc diffussion test (CDDT) the zone of inhibition diameter was measured objectively by measuring scale . Combined disc diffussion test (CDDT) was found to be a cost effective and convenient MBL screening method in the clinical microbiology laboratories.3 In this study 46 (29.48%) isolates of *Pseudomonas aeruginosa* were MBL Producers. Which is in accordance with other Indian studies like Ami varaiya 6 (20%), John 7 (27.7%) and Arunava kali14 (22.4%). All the MBL negative isolates showed 100% sensitivity to polymxyin B, colistin, carbapenams, piperacillin tazobactam ,Cephalosporins and showed 67.2 % sensitivity to Amikacin and 63.2 % sensitivity to Ciprofloxacin. Among the MBL positive isolates from burns wound infection, maximum sensitivity were observed for polymyxin B (100%), colistin(100%) followed by piperacillin tazobactam (34.7%).6 High resistance were observed in Aminoglycosides , Flouroquinolones, Cephalosporins and Carbapenams. Similar observations were seen in Senthamarai 8, Hemalatha 11, Bose 13,and Shweta1. MBL producing *Pseudomonas aeruginosa* showed high resistance to most of the antibiotics, But all the isolates were susceptible to Polymyxin B(300 units) and Colistin (10 µg). As the treatment option for such isolates are very limited, early detection of MBL producers with their sensitivity pattern is necessary for administration of specific treatment to prevent the development and dissemination of multi drug resistant strains.

Conclusion:

MBL production is an important mechanism of carbapenem resistance among *Pseudomonas aeruginosa* . In our study, Double disc synergy test (DDST) and *Imipenem- EDTA*Combined Disc Diffusion Test (CDDT) was used as a cost effective phenotypic method for detection of MBL production. The early detection of MBL producing *Pseudomonas aeruginosa will* be useful for effective management of burns wound infection and to implement a fully operational scientifically designed antibiotic policy for burns unit. Antibiotic stewardship programme should be made which is the main stay in the management of burns wound infection and helps to prevent the development and dissemination of these multi drug resistant strains to achieve the ultimate objective of decreasing infection related morbidity and mortality in burns wound infection.

References:

1. S.Shweta , C.Hans, L.K. Makhiljaand R.K.Mahajan, Prevalence of Extended Spectrum beta lactamases and Metallo beta lactamases in bacterial isolates from burn patients. Int.J.Curr.Microbiol.App.Sci (2014) 3(I):529-53.
2. S.Buchende, DK.Mendiratta,V.Deotale, P.Narang, Comparsion of disc and MIC reduction methods with Polymerase chain reaction for the detection of metallo beta lactamase in *Pseudomonas* aerugiosa. Indian J. Med Microbiol (2012) 30(2) :170-4
3. B.Behera, P Mathur A.Das, A.Kapil, V.Sharma, An evaluation of four different phenotypic techniques for detection of metallo beta lactamase producing *Pseudomonas aeruginosa*. Indian J. Med Microbiol (2008) 26(3) :233-37
4. Mackie &McCartney, Practical Medical Microbiology 14 th edition .
5. Clinical And Laboratory Standards Institute, Performance standards for antimicrobial susceptibility testing; 21st informational supplement M100-S21;Wayne,PA ;2011
6. Ami Varaiya, Nikhil Kulkarni, Manasi Kulkarni, Pallavi Bhalekar & Jyotsana Dogra, Incidence of metallo beta lactamase producing *Pseudomonas aeruginosa* in ICU patients. Indian J Med Res 127, April 2008, pp 398-402.
7. S.John, Balagurunathan R, Metallo beta lactamase producing *Pseudomonas aeruginosa* and Acinetobacter baumannii. Indian J. Med Microbiol (2011) 29(3) :302-304.
8. Senthamarai s, Suneel Kumar reddy, A Sivasankari, et al Resistance pattern of *Pseudomonas aeruginosa* in a tertiary care hospital of Kanchipuram, Tamilnadu, India. Journal of clinical and Diagnostic Research 2014 May, Vol 8(5) DO 30 – DO 32.
9. A Manoharan, S Chatterjee, D Mathai, SARI study group, Detection and characterization of metallo beta lactamases producing *Pseudomonas aeruginosa*. Indian J. Med Microbiol (2010) 28(3) :241-4.
10. M.J.C Noyal, G.A. Menezes, B.N.Harish, S.Sujatha & S.C. Parija , Simple screening tests for detection of carbapenamases in clinical isolates of non fermentative Gram – negative bacteria. Indian J Med Res 129, June 2009, pp 707-712
11. Hemalatha V, Uma sekar & Vijaylakshmi Kamat, Detection of metallo beta lactamases producing *Pseudomonas aeruginosa* in hospitalized patients. Indian J Med Res 122, August 2005, pp 148-152
12. M. Purohit, DK.Mendiratta, V.Deotale, M Madhan , A Manoharan, P.Narang, , Detection of metallo beta lactamases producing Acinetobacter baumannii using microbiological assay, disc synergy test and PCR. Indian J. Med Microbiol (2012) 30(4) :456-61
13. Seema Bose, Atindra Krishna Gosh, Rekha Barapatre, Incidence of metallo beta lactamases producing *Pseudomonas aeruginosa* in burns ward of a tertiary care rural hospital. Int. J. Biomedical Research 3(05)(2012) 233-238
14. Arunava Kali, Sreenivasan Srirangaraj, Shailesh Kumar, Hema , et al Detection of metallo beta lactamases producing *Pseudomonas aeruginosa* in intensive care units. Australian Medical Journal 2013, 6, 12, 686-693