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INCIDENCE OF METALLO BETALACTAMASES PRODUCING *PSEUDOMONAS AERUGINOSA* IN BURN WARD OF A TERTIARY CARE RURAL HOSPITAL

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ABSTRACT

Background: Prevalence of *Pseudomonas aeruginosa* infection is very common among indoor burn patients. *Metallo-β-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 β lactams. The drug resistance due to MBL has a potential for rapid spread to other micro organisms.

Method: A prospective study was conducted over a period of 8 months. All the *Pseudomonas aeruginosa* were isolated from infected burn wounds of patients admitted in burn ward. Imipenem resistant isolates were further tested by Hodge test, disk potentiation test, double disk synergy test (DDST) and MBL E test.

Results: Out of total 140 *Pseudomonas aeruginosa* isolates, 42 (30%) were imipenem resistant, among which, 20 (14.28%) were non MBL producers and 22 (15.71%) were MBL producers. MBL producers were more resistant to commonly used antibiotics than non MBL producers. All isolates were sensitive to colistin (10µg) and polymyxin B (300 µg).

Conclusion: Incidence of MBL producing *Pseudomonas aeruginosa* infection is relatively high in our set up. Simple methods for MBL production should be done routinely. Patients infected with MBL producing organisms should be promptly isolated and antibiotic stewardship programme should be made to prevent spreading of resistance.

Keywords: Pseudomonas aeruginosa, Metallo–β–lactamases, Hodge test

1. Introduction

Bacterial infections are a common cause of mortality and morbidity in burn patients.[1] Microorganisms infecting burn patients are either endogenous (normal flora of the patients) or exogenous (from the environment and from health care personnel).Exogenous organisms from the hospital environments are generally more resistant to antibiotics than endogenous organisms. Gram negative organisms have long been known to cause serious infections in burn patients and outbreak of cross infection is a major challenge to burn unit.[2]

Pseudomonas aeruginosa has been described as the most common and most serious cause of infection in burn patients. In this study, we aimed to determine *metallo-β-lactamases* (MBL) production in *Pseudomonas aeruginosa* isolated from infected burn wounds, in a tertiary care rural hospital. This enzyme causes Imipenem resistance and increase the mortality rate of burn patients. Timely identification of MBL producing strains and strict isolation of patients, prevent further spread of MBL producing genes to other gram negative bacteria. For that, it is essential for carbapenem resistant isolates to be screened for MBLs.[3, 4, 5]

This study has been conducted in a health care facility situated in rural India. Even in this population, the incidence of Metallo-β-lactamases (MBL) producing organism was considerably high. Routine testing of MBL production by microorganisms should be included in the microbiology laboratories.

2. Material and Methods

2.1 Ethics committee approval – The study was approved by the Institutional ethical committee. (Reference no – PMT/ RMC/ RC/2010/522)

Samples were collected from the indoor burn patients after removal of dressing and topical antibiotics and cleaning the wound with sterile gauze piece. Specimens were obtained with the end of sterile cotton swabs moistened with sterile saline. The swab was moved over a minimum one centimetre area of the open wound and enough pressure was applied to the tip of the swab to release fluid from the wound surface. Sample was also taken from the edge of the wound site showing signs of infection.[6,7] Swabs were transferred to microbiology laboratory immediately for Gram staining, culture and antibiotic sensitivity test.

We isolated 140 *Pseudomonas aeruginosa* from wound swabs of burn patients over a period of nine months. The identification of isolates up to species level was done in the microbiology department as per the conventional methods[8]. The antibiotic susceptibility tests of the isolates were done by the disk diffusion method following CLSI guidelines.[9] Antibiotic disks used for this study were – Imipenem (10µg), piperacillin/ tazobactam, (100/10 µg), netilmycin (30µg), ticarcillin (75µg), amikacin (30µg), ceftazidime (30µg), ciprofloxacin(5µg),colistin (10µg), cefepime (30µg), cefoxitin (30µg), Polymyxin B (300 units) and aztreonam (30µg). All the antibiotic disks were procured from Himedia pvt ltd, India. All imipenem resistant *Pseudomonas aeruginosa* (by disk diffusion method) showed high MIC (minimum inhibitory concentration) values to imipenem ranging from 16 – 128 µg/ml (by E test strip (AB bioMerieux).

Imipenem resistant *Pseudomonas aeruginosa* were tested by Hodge test, disk potentiation test, using imipenem and imipenem– EDTA (ethylene diamine tetra acetic acid) disks respectively, double disk synergy test (DDST) and MBL E test (AB bioMerieux).[10, 11, 12, 13] ATCC 27853 *Pseudomonas aeruginosa* was used as negative control.

2.2. Statistical Methods: The comparative statistical analysis for all phenotypic methods for detection of MBL were done.[14] All 17 isolates, which were positive for MBL production detected by Hodge test were also showed positivity with Disk potentiation test, DDST and MBL E test. Similarly, all 20 isolates, showing positive MBL production with disk potentiation test, were also positive for the same with DDST and MBL E test. Considering MBL E test as gold standard for detection of MBL production, positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity were calculated using following formulae –

Positive predictive value = True positive / (True positive + False positive) × 100

Negative predictive value = True negative / (False negative + True negative) × 100

Sensitivity = True positive / (True positive + False negative) × 100

Specificity = True negative / (True negative + False positive) ×100

3. Observations and Results

Total 140 *Pseudomonas aeruginosa* were isolated from burn ward of a rural tertiary care hospital, over a period of nine months.(Figure 1). Out of 140 *Pseudomonas aeruginosa* isolates, 42(30%) were imipenem resistant (Table 1, Figure 2) and 22(15.71%) were MBL producers (Table 1).

MBL production was detected by performing Hodge test (Figure 3), Disk potentiation test; DDST, (Table 2, Figure 4) and MBL E test (Figure 5).

All the MBL producers were polymyxin B and colistin sensitive and resistant to most of the antibiotics used for antibiotic susceptibility test. Only one non MBL producer *Pseudomonas aeruginosa* was resistant to all ten antibiotic disks, used for this study, except colistin and Polymyxin B (Table 3).

Considering MBL E test as standard for detection of MBL production, the sensitivity of Hodge test, Disk potentiation test and DDST were 77%, 90%, and 100% respectively. Specificity of all the above mentioned tests was 100%, indicating all these tests can identify 'true negative' isolates for MBL production.

Figure 1 – Colonies of *Pseudomonas aeruginosa* on nutrient agar, showing

diffusible, green pigment.

Figure 2 – Imipenem resistant *Pseudomonas aeruginosa* by disk diffusion method

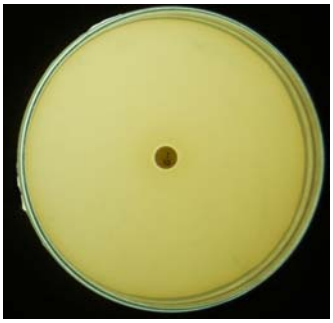

Table 1 – Distribution of Imipenem resistant strains and MBL producing strains of *Pseudomonas aeruginosa* (n = 140)

Total no of *Pseudomonas aeruginosa* isolates	Total number of Imipenem resistant strains	Total number of MBL producing strains
140	42 (30 %)	22(15.71%)

Table 2 – Results of Hodge test, Disk potentiation test, DDST and MBL E test (n = 42)

Hodge Test	Disk Potentiation Test	DDST	MBL E Test
17 (40.47%)	20 (47.61%)	22 (52.38%)	22 (52.38%)

Figure 3 – Hodge test positive.

Figure 4: Disk potentiation test (A) & DDST (B) positive

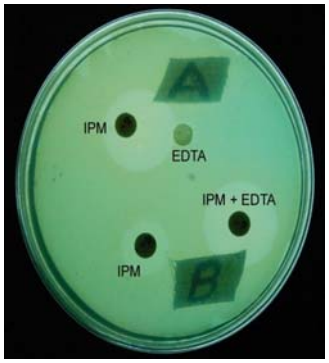

Figure 5: The phantom zone shown in the figure indicating MBL E Test positivity

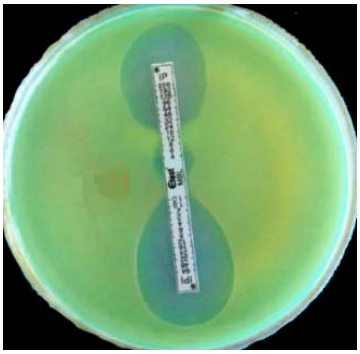

Table 3 - Antibiotic susceptibility pattern of MBL producing and non MBL producing, imipenem resistant *Pseudomonas aeruginosa*

Antibiotics	Number & % sensitivity of MBL producers (n=22)	Number & % sensitivity of MBL non producers (n=20)
Piperacillin/Tazobactam	0 (0%)	13(65%)
Netilmycin	1(4.5%)	9(45%)
Ticarcillin	0 (0%)	3(15%)
Amikacin	1 (4.5%)	12(60%)
Ceftazidime	0 (0%)	4(20%)
Ciprofloxacin	0 (0%)	9(45%)
Cefepime	0 (0%)	1(5%)
Cefoxitin	0 (0%)	1(5%)
Polymyxin B	22(100%)	20(100%)
Aztreonam	0 (0%)	4(20%)
Colistin	22(100%)	20(100%)

5. Discussion

Pseudomonas aeruginosa is frequently responsible for outbreak of hospital acquired infection worldwide. Infection in burn patients is one of the most challenging concerns for the burn team involved in patient care. High rate of infection in burn patients is due to loss of protective barrier of skin and presence of devitalized tissues supporting the growth of microorganisms. This also inhibits penetration of systemically administered antibiotics. MBL producing organisms are more resistant to commonly used antibiotics, especially carbapenems.[2] Resistance to carbapenems is due to increased efflux system, decreased outer membrane permeability, alteration of penicillin binding proteins and production of carbapenem hydrolyzing enzymes.[15]

The first MBL, encoded on a plasmid, IMP–1 (for “active on imipenem”), was discovered in

Japan in 1988.[16] In India, MBL producing *Pseudomonas aeruginosa* was first reported in 2002.[17]

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-β-lactamases"), GIM (for "German imipenemase") and SIM (for "Seoul imipenemase") families. SPM–1(for "Sao Paulo Metallo-β-lactamases"), was a new family and first time isolated in *Pseudomonas aeruginosa*. After detected primarily in *Pseudomonas aeruginosa*, it was found in other Gram negative bacteria also.[18]

The MBLs need one or more divalent cataions at the active site, e.g., EDTA, $CuCl_2$ etc.[19]

There are several phenotypic and genotypic methods, available for detection of MBL. At present, no Clinical Laboratory Standard Institute (CLSI) guideline is available for the detection of MBL producing organisms. Genotypic methods of detection of MBL producing organisms give specific and accurate results,[20] but due to cost constraints, it is of limited use, especially in a health care institute serving rural population. In our study, we adopted simple phenotypic methods to detect MBL producing *Pseudomonas aeruginosa*, isolated from patients from burn ward and found these methods quite satisfactory.

We used sample swabbing method for collection of samples from burn patients. For this purpose, mainly two methods are there – surface swabbing and biopsy. Surface swabbing, if properly done, is very convenient and effective method. Viable, unburned tissue biopsy from a burn patient for diagnosis of microbial invasion, confirms invasive burn wound infection. But recently, value of this costly, laborious and invasive method has been questioned.[6]

Bahar M A et al observed that there was a good correlation between non invasive surface swab and invasive biopsy for identification of pathogens over the surface and within the burn wound. [7]

We used improved Hodge test by adding 50 mM of zinc sulphate on imipenem disk. According to some workers, testing for ceftazidime resistant isolates for MBL production, are more satisfactory, because Gram negative bacilli were inhibited by low concentration of imipenem and were difficult to detect.[21,] In our study, we used DDST and Disk potentiation test with imipenem and imipenem–EDTA. We found two non MBL producing isolates which were ceftazidime resistant. Sometimes ceftazidime resistance in MBL producers follows some other mechanisms. [1] In such cases, use of imipenem disk is useful.

In our study, out of 42 imipenem resistant *Pseudomonas aeruginosa,* only 22 strains were MBL producers. Carbapenem antibiotics penetrate the outer membrane of *Pseudomonas aeruginosa* through a porin protein, OprD2. It was noted by others that the loss of OprD porin in *Pseudomonas aeruginosa* was one of the important mechanism causing imipenem resistance, other than carbapenemase.[22, 23, 24]

We isolated 22(15.71%) *Pseudomonas aeruginosa*, which were MBL producers. This was similar to the result observed by Navaneeth BV et al[17] (12% MBL producers) and different from the studies conducted by Varaiya A et al[25], Behera B et al[26] and Gladstone P et al.[27] Another group of researchers reported 50% MBL producing *Pseudomonas aeruginosa* in their study.[28]

The most worrisome problem with MBL producing organisms is that, they are resistant to commonly used broad spectrum antibiotics, including amino glycosides, fluoroquinolones and β lactams.[25] Initially, MBLs, such as SPM, GIM and SIM remained confined to their countries of origin, but VIM and IMP were detected worldwide, spreading from *Pseudomonas aeruginosa* to enterobacteriaceae.[20] MBL producing *Pseudomonas aeruginosa* showed high resistance to ten antibiotics disks used here, except colistin (10µg) and polymyxin B (300 units), to which, all the isolates were susceptible. (Table 3)

Conclusion

This study documents that there are relatively high incidence of patients infected with MBL producing organisms, in burn ward of our setup. All clinical microbiology laboratories must routinely test for MBL production. There are several screening tests for detection of MBL production but no single test when used alone is specific for the same.[13] Rizvi et al[29] found DDST using EDTA better than Hodge test. Lee K et al also found that Hodge test was a simple screening method for MBL producing isolates, but occasional isolates showed false negative result.[10] In our study, we found DDST and MBL E test both were equally

sensitive and easy to perform. Isolation of the patients, infected with MBL producing organisms should be done immediately. Strict safety precaution, such as, use of gowns, gloves and hand washing, before and after each patient visit should be followed. Old antimicrobials, i.e., polymyxin B and polymyxin E (colistin) are again coming back in use and have been successfully used by many workers against multi drug resistant *Pseudomonas aeruginosa* infection.[30] However; some researchers reported emergence of colistin resistant organisms in their study.[31] The judicious selection of antibiotics to treat such patients should be implemented.

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