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ORIGINAL ARTICLE

A STUDY OF METALLO-BETA-LACTAMASE PRODUCING PSEUDOMONAS AERUGINOSA IN BLOOD SAMPLES OF BURNED PATIENTS

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ABSTACT: BACKGROUND: Septicaemia is a life threatening complication of severely burned patients. Among many organisms invading blood stream Pseudomonas aeruginosa is a well-known for its powerful antibiotic resistance mechanisms which increasingly limit the choices for treatment. Among many such resistance mechanisms it is the metallo-beta-lactamase (MBL) which confers resistance to Carbapenem group of antibiotics, one of the final resorts to fight them. The present study was undertaken to detect MBL producing P. aeruginosa using phenotypic method from blood samples of burned patients as well as to know their drug sensitivity pattern. **MATERIALS AND METHODS**: For this purpose 67 Pseudomonas aeruginosa isolates from blood samples of admitted burned patients were subjected to susceptibility testing to antipseudomonal drugs by disc diffusion test and those found to be Carbapenem resistant were subjected to Imipenem - EDTA combined disk synergy test for MBL detection. **RESULT:** Out of 67 isolates of P.aeruginosa, 19 (28.4%) were found to be Carbapenem resistant and 11 (16.4%) were MBL producers. A particularly important feature was that the MBL producers were highly resistant to the antibiotics tested than the non-producers. However all of them were susceptible to Colistin and Polymixin B. **CONCLUSION:** This study has made us to think that a constant vigil and careful selection of antibiotics are necessary to keep prevalence of MBL producing P.aeruginosa in check. The accurate identification and reporting of MBL producing P. aeruginosa will aid infection control practitioners in preventing the spread of these multidrug-resistant isolates.

KEYWORDS: Septicaemia, Burned patients, Metallo-beta-lactamase, Pseudomonas aeruginosa.

INTRODUCTION: Sepsis remains a significant factor affecting morbidity and mortality in burned patients.[1] It has been estimated that 75% of all deaths following thermal injuries are related to infections.[2] Sepsis in burns is commonly due to bronchopneumonia, pyelonephritis, thrombophlebitis, or invasive wound infection. These infections are largely hospital-acquired and the infecting pathogens differ from one hospital to another.[3]

However, one of the pathogens which are a common invader in burn wards is Pseudomonas aeruginosa. Unfortunately P. aeruginosa is also notoriously known worldwide for developing antimicrobial resistance, thus jeopardizing the treatment option and sealing the patient's fate. Like many other gram negative bacteria which have developed multi drug resistance, Carbapenems became the mainstay of therapy to treat this green pathogen.

However million dollars of pharmaceutical research were undone by swift strokes of genetic evolution in P. aeruginosa, and they developed Carbapenem resistance.

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To resist carbapenems, these gram negative bacilli have started producing two types of enzymes: serine carbapenemases, and metallo-beta-lactamases (MBLs).[4] These enzymes can hydrolyze not only carbapenems but many β lactams as well. [5] The genes responsible for production of MBLs lie on a plasmid, and hence can be horizontally transferred easily, efficiently and rapidly to other bacteria.[6]

So, it is imperative to keep a hawk-eyed watch on the developing resistance pattern of Pseudomonas aeruginosa, especially in the burn wards as this can only keep the increasing burden of infections in check, which is a major cause of mortality in these patients. Our main aim was to have inkling that, how much of the burn wards of our apex institution has been invaded by this bug with a metallic gun in its pocket, as well as to find its susceptibility pattern to the anti pseudomonal drugs.

MATERIALS AND METHODS: The prospective, observational, hospital based study was performed on admitted burned patients in accordance with the ethical standards of the responsible institutional committee on human experimentation for a period of one year.

In this study blood culture was performed from the patients with presence of signs of sepsis and suspected blood stream infection whether primary or secondary to infection in another site. A total of 67 P. aeruginosa were isolated from blood samples. Susceptibility testing to anti pseudomonal drugs of isolated P.aeruginosa was done on Mueller Hinton agar with commercially available discs by Kirby- Bauer method according to CLSI guidelines.[7] Standard strains of P. aeruginosa ATCC 27853 was used as control.

Following antibiotic discs were used: Amikacin-30 µg, Gentamicin-10 µg, Tobramycin-10 µg, Ofloxacin-5 µg, Ciprofloxacin-5 µg, Cefoperazone-75 µg, Ceftazidime-30 µg, Cefoperazone-75 µg plus Sulbactum-30 µg, Cefepime- 30 µg, Carbenicillin-100 µg, Piperacillin-100 µg, Piperacillin-100 µg plus Tazobactum-10 µg, Imipenem-10 µg, Meropenem-10 µg, Polymyxin B-300 units and Colistin-10 µg. Those found to be Carbapenem resistant were further subjected to tests for MBL detection.

There are various recommended techniques for phenotypic determination of MBL, like Imipenem - EDTA Combined disk synergy test, Imipenem - EDTA Double disc synergy test, Ceftazidime - EDTA Combined disk synergy test, Ceftazidime - EDTA Double disc synergy test, E-test strip and microdilution technique for determining MIC of Imipenem.[8,9,10] We took up the Imipenem - EDTA Combined disk synergy test.

In this test, organisms were inoculated on the MHA plates as recommended by CLSI.[7] A 0.5M EDTA solution was prepared by dissolving 186.1 gm of Disodium EDTA.$2H_2O$ in 1000 ml of distilled water and its pH was adjusted to 8.0. This solution was sterilized by autoclaving. Then two 10µg Imipenem discs were placed on test organism inoculated MHA and 5µl EDTA was added to one Imipenem disc. After 16 hours of incubation at 35°c, the zones of inhibition around both the discs were measured. The strains with enhanced zones of at least 7mm around EDTA impregnated Imipenem discs compared to the Imipenem disc alone were identified as MBL producing Pseudomonas aeruginosa.

RESULTS: A total of 67 isolates of P.aeruginosa from blood stream of burned patients have been included in this study. Out of such 67 isolates 19 (28.4%) were found to be resistant to Carbapenem. These resistant strains were further subjected to MBL detection by Imipenem - EDTA combined disk synergy test. In this test 11 isolates turned out to be MBL producers comprising 16.4% of total isolated strains and 57.9% of the Imipenem resistant strains.

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Antibiotics	Numbers (%)	
	Sensitive	Resistant
Amikacin	3 (27.3)	8 (72.7)
Gentamicin	4 (36.4)	7 (63.6)
Tobramycin	5 (45.5)	6 (54.5)
Ciprofloxacin	3 (27.3)	8 (72.7)
Ofloxacin	2 (18.2)	9 (81.8)
Ceftazidime	0 (0)	11 (100)
Cefoperazone	1 (9.1)	10 (90.9)
Cefoperazone- Sulbactum	4 (36.4)	7 (63.6)
Cefepime	0 (0)	11 (100)
Piperacillin	0 (0)	11 (100)
Carbenicillin	0 (0)	11 (100)
Piperacillin- Tazobactum	2 (18.2)	9 (81.8)
Imipenem	0 (0)	11(100)
Meropenem	0 (0)	11 (100)
Polymixin B	11 (100)	0 (0)
Colistin	11 (100)	0 (0)

Table 1: Susceptibility pattern of MBL producing Pseudomonas aeruginosa (n=11)

Antibiotics	Number (%)	
	Sensitive	Resistant
Amikacin	29 (51.8)	27 (48.2)
Gentamicin	31 (55.4)	25 (44.6)
Tobramycin	38 (67.9)	18 (32.1)
Ciprofloxacin	24 (42.9)	32 (57.1)
Ofloxacin	17 (30.4)	39 (69.6)
Ceftazidime	16 (28.6)	40 (71.4)
Cefoperazone	24 (42.9)	32 (57.1)
Cefoperazone- Sulbactum	37 (66.1)	19 (33.9)
Cefepime	36 (64.3)	20 (35.7)
Piperacillin	15 (26.8)	41 (73.2)
Carbenicillin	18 (32.1)	38 (67.9)
Piperacillin- Tazobactum	39 (69.6)	17 (30.4)
Imipenem	48 (85.7)	8 (14.3)
Meropenem	48 (85.7)	8 (14.3)
Polymixin B	56 (100)	0 (0)
Colistin	56 (100)	0 (0)

Table 2: Susceptibility pattern of MBL nonproducing Pseudomonas aeruginosa (n=56)

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DISCUSSION: Despite recent advances in the use of topical and parenteral antimicrobial therapy and the practice of early tangential excision, bacterial infections remain a major problem in the management of burn victims today.[11] Till now sepsis remains a significant factor affecting morbidity and mortality in burned patients and Pseudomonas aeruginosa is a well-known invader from hospital environment in such critically ill patients.

It has been isolated from environments as diverse as water, jet plane fuel and disinfectant solutions due to its ability to utilize many different organic compounds and survive in the apparent absence of nutrients.

This all-pervading organism has also invented multiple mechanisms of antibiotic resistance, thus leaving in our hands but a few choices to treat the patients. One of the most dangerous among them is metallobetalactamase (MBL). The genes responsible for production of MBLs are VIM and IMP. Most of these genes are found as gene cassettes in integrons. In most instances, the MBL genes are fused with aacA4 genes which codes for aminoglycoside resistance, further compromising both antibiotic regimens. MBL in such Gram negative bacilli is becoming a therapeutic challenge, as these enzymes usually possess a broad hydrolysis profile that includes all β-lactam antibiotics including Carbapenems.

Since there are no standard guidelines for detection of MBL, different studies have reported the use of different methods. PCR analysis is the gold standard method for the detection of MBL production, but it is not feasible in routine microbiology laboratory. In different studies Imipenem - EDTA Combined disk synergy test was found to be a sensitive method for MBL detection.[12] So this method has been used in study for MBL detection.

In various studies across the world, varying resistance (4-60%) has been seen towards Carbapenems.[13,14] In this study 28.4% of isolated P.aeruginosa were carbapenem resistant. P. aeruginosa producing MBL was first reported from Japan in 1991.[15] In 2002 from India, Navneeth et al[16] first reported MBL production in P. aeruginosa to be 12 per cent. Since then, the incidence of MBL production in P. aeruginosa has been reported to be 10-30 per cent from various clinical specimens across the country.[17]

This study has revealed the fact that 16.4% of isolated P.aeruginosa from blood sample of burned patients were MBL producers. The result was almost similar to the study conducted by Varaiya A et al showing isolation rate of MBL producer to be 20.8%.[18]

MBL producing isolates usually show high level of resistance to all β-lactam antibiotics, aminoglycosides, tetracycline, and fluoroquinolones though they remain sensitive to polymyxin B. In this study, it was evident that MBL producing strains were more resistant to the tested antibiotics in comparison to the non MBL producers. Very few antibiotics such as Polymixin B and Colistin were active against them. This was in concordance with the study report of Bose S et al.[19]

MBL producing isolates are associated with a higher morbidity and mortality. Moreover given the fact that MBLs will hydrolyze all classes of β-lactams and that we are several years away from the development of a safe therapeutic antibiotic; their continued spread would be a clinical disaster. The occurrence of an MBL positive isolate poses not only a therapeutic problem but is also a serious concern for infection control management.

As a result of being difficult to detect, such organisms pose significant risks particularly due to their role in unnoticed spread within institutions and their ability to participate in horizontal MBL gene transfer, with other pathogens in the hospital. Hence the early detection of MBL-producing

isolates would be important for the reduction of mortality rates for patients infected with MBL producing isolates and also to avoid the intra hospital dissemination of such multi drug resistant strains. For this purpose the development of simple screening test designed to detect MBL production will be a crucial step towards large scale monitoring of this emerging resistant determinant.

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