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PHENOTYPIC DETECTION OF METALLO BETA LACTAMASE PRODUCING PSEUDOMONAS AERUGINOSA IN WOUNDS

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ABSTRACT

Pseudomonas aeruginosa, an important opportunistic pathogen causing a wide range of acute and chronic infections.The present study was undertaken to detect the Metallo β lactamase in isolated of *Pseudomonas aeruginosa* which was isolated from wound infection and evaluated their susceptibility patterns.The presence of the MBL enzyme was detected by phenotypic confirmatiory tests are IMIPENEM-EDTA combined disc synergy test and Imipenem EDTA double disk synergy test .Out of 161 sample which were culture 39(24.2%)yielded the growth of Pseudomonas aeruginosa. Among the 39 Pseudomonas aeruginosa isolates 14(35.8%) were MBL produced P.aeruginosa and were also resistant to Ceftazidime/Clavulonic acid (CAC),Ceftazidime(CAZ), Amikacin(AK),Ciprofloxacin(CIP),Gentamicin(GEN).In our study out of 39 isolates, 14(35.4%) isolates are resistant to carbapenem and confirmed by disk potentiation test. The early detection may help in appropriate antimicrobial therapy and avoid the multidrug resistant strains.we recommend a routine surveillance on antibiotic resistance in every clinical microbiology laboratory in order to aid infection control.

KEYWORDS: ***Pseudomonas aeruginosa,*** **Metallo β lactamase, Imipenem EDTA double disk synergy test and Ceftazidime**.

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INTRODUCTION

Pseudomonas aeruginosa an important opportunistic pathogen causing a wide range of acute and chronic infections. It is one of the most common non- fermenting pathogen involved in nosocomial infections[1]. Infections due to *P.aeruginosa* have more prevalent among patients with burns wounds, cystic fibrosis, acute leukemia, organ transplants and intravenous drug-addiction[2]. *P.aeruginosa* is multifactorial with mutations in gene encoding porins, efflux-pumps, penicillin-binding protein and chromosomal –β lactamase. All contributing to survive in varied environment and resistance against multiple antimicrobial drugs. *P.aeruginosa* producing metallo-β lactamases (MBL) is an issue of public health concern[3]. The first plasmid –mediated MBL was reported in *P. aeruginosa* in Japan in 1991 and it is also found in Europe , Australia, South America and NorthAmerica[4]. Metallo beta lactamase (MBL) belongs to a group β-lactamase which requires divalent cations of zinc as cofactors for enzyme activity[5]. These have potent hydrolyzing activity against carbapenem and other β-lactam antibiotics. Based on molecular studies carbapenem hydrolyzing enzymes are classified into four groups A, B,C and D.These carbapenemase are classB metallo β-lactamases IMP,VIM, Class D-oxacillinnases OXA23 to OXA27 and ClassA clavulanic acid inhibitory enzymes SME, NMC, IMI, KPC [6].The IMP and VIM genes are usually part of an integron structure and are carried on horizontally transferable plasmids and also be part of the chromosome[7]. Resistance to carbapenem is due to decreased outer membrane permeability, increased efflux systems, alteration of penicillin binding proteins and carbapenem hydrolyzing enzymes. In recent years, the MBL genes have spread from *P.aeruginosa* to *Enterobactericeae* and resistance to different group of antimicrobial agents. Therefore the local surveillance program is needed at each center, as the knowledge of local resistant patterns which is vital for selecting appropriate agents for treating infection. So, the present study is about phenotypic detection and antibiotic sensitivity of MBL producing *Pseudomonas* from wound swabs. It helps an effective antibiotic strategy and to plan a proper hospital infection control strategy to prevent the spread of these strains.

MATERIALS AND METHODS

This descriptive study was conducted between Dec2013-MAY2014 in the department of Microbiology, SLIMS. Out of 39, *P.aeruginosa* were obtained from pus samples of wound infection cases. Most of the specimens were received from patients admitted in Medical and surgical wards. The cases which yielded a growth of *P.aeruginosa* from culture wound swab on three different media namely, Nutrient agar, blood agar and MacConkey agar and incubated at 37^0C. The following characteristics were used to identify *P.aeruginosa* isolates ; Gram-negative bacilli, colonies with characteristic pigment(blue-green) and a special odor ,positive catalase and oxidase tests, oxidation of glucose on OF medium and growth at 42^oC.The isolates were antimicrobial sensitive which was determined using Kirby Bauer disc diffusion method. The antbiotic disk AT,COT,CTR,CL,CTR CAZ, CIP, G, AK, PIT, I. The results were recorded and interpreted as per as CLSI guidelines and *P.aeruginosa* ATCC 27853 was used as a negative control for MBL positive control.

PHENOLYTIC DETECTION OF MBL-PRODUCING ISOLATES Imipenem –EDTA Combined Disc Synergy Test (CDST)

0.5M EDTA was prepared with distilled water and sterilized by autoclaving. Imipenem disks were supplemented with EDTA by dispensing 10µl of this solution to each imipenem disk. Imipenem-EDTA combined disk method (CDT) was performed as described by Yong et al. A lawn culture of test isolates was prepared. After allowing it to dry for five minutes, two imipenem discs, one with 0.5 M EDTA and the other a plain imipenem disc, were placed on the surface of agar plates approximately 30mm apart. The plates were incubated overnight at

37°C. An increase in zone diameter of ≥ 7mm around imipenem+EDTA disk in comparison to imipenem disk alone indicated production of MBL

Imipenem-EDTA double disk synergy test (DDST)

Imipenem-EDTA double disk synergy test (DDST) was performed as described by Lee et al. Test organisms were inoculated on to the plates with Mueller Hinton agar as recommended by CLSI. An imipenem (10µg) disk was placed 20mm centre to centre from a blank disk containing 10µL of 0.5 M EDTA (750µg). Enhancement of the zone of inhibition in the area between imipenem and EDTA disk in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result for MBL production.

RESULTS

Out of 161 pus samples which were cultured, 39(24.2%) yielded the growth of *Psuedomonas aeruginosa.* 28 samples yielded pure growth *Psuedomonas aeruginosa* while 11 isolated were mixed culture with *E.coli, Staph. aureus, Klebsilla pneumonia*. Among the isolates from the inpatient department 29 (74.3%) isolated from the Orthopaedics ward followed by 10 (25.6%) from surgery ward. The resistance of *Psuedomonas aeruginosa* was noted as follows CAZ (Ceftazidime) 11(28.2%), Ceftazid/Clavulonic acid(CAC) 9(23.0%), Ceftriaxone(CTR) 7 (17.9%), Amikacin(AK) 4(10.2%), Ciprofloxacin(CIP) 3(7.6%), Tetracycline(T) 2(5.1%), Gentamycin(GEN) 1(2.5%) . Among the 39 isolates Psuedomonas aeruginosa 14(35.8%) isolates were metallo β lactamase producer and were also resistant to CAZ, CAC ,CTR,AK and CIP.

Table 1
Isolation of MBL producing Pseudomonas aeruginosa from out patients and inpatients

Inpatients (28)		Outpatients(11)	
MBL	Non- MBL	MBL	Non-MBL
11 (39.2%)	17(60.7%)	3 (27.2%)	8 (72.7%)

Out of 39 subjects who showed the growth of MBL produced Psuedomonas aeruginosa 11(39.2%) were inpatients and 3 (27.2%) were outpatients.

Table 2
Isolation of MBL producing Pseudomonas aeruginosa from Males and Females

Male (34)		Female (5)	
MBL	Non- MBL	MBL	Non-MBL
13 (38.2%)	21 (61.7%)	1 (20.0%)	4 (80.0%)

Out of 39 isolates 13 (38.2%) MBL produced P.aeruginosa were male patients and 1(20.0%) were female patients.

Table 3
Isolation of MBL producing Pseudomonas aeruginosa From Diabetic patients and Non Diabetic patients

Diabetic patients(29)		Non diabetic patients(10)	
MBL	Non- MBL	MBL	Non-MBL
12(41.3%)	17 (58.6%)	2(20.0%)	8 (80.0%)

Out of 39 isolates 12(41.3%) MBL producing P.aeruginosa were diabetic patients and 2(20.0%) were non- diabetic patients

DISCUSSION

The emergence of antibiotic resistant bacteria is threatening the effectiveness of many antimicrobial agents. It has increased the hospital stay of the patients,thus leading to an increased economic burden on them. We have isolated 39*Pseudomonas aeruginosa* from wound swabs during the study period. In various studies across the world, varying resistance (4-60%) has been towards Imipenem and meropenem[8].we found 35.8% resistance to imipenem and meropenem[9]. In 2002 from india, first reported MBL production in *P.aeruginosa* to be 12 %[10]. Since,then the incidence of MBL production in *P.aeruginosa* has been reported to be 10-30% from various clinical specimens across the country. The percentage of mbl in the imipenem resistant isolates (35.8%).this suggested that the carbapenem resistance in *P.aeruginosa* was mediated predominantly via MBL production .A similar finding was observed by the SARI study group and by Behara etal. In the present study 3 isolates (21.4%) out of 14 MBL were resistant to all the6 antibiotics which were tested the presence of MBL in the pan drug resistant isolated was already observed by jaykumari.S[11] . In our study MBL producing *P.aeruginosa* more in inpatients as compared the out patients. It is due to the duration of the hospital stay was directly proportional to higher prevalence of the infection. Since the rate of isolation of the organism was higher indoor patients than in outdoor patients. TABLE-1A similar observation was made by Shampa Anupurba et al[12]. MBL producing *P.aeruginosa* more in male(38.2%) as compared with the female . Males can be explained by the greater number of cases from Surgery and Orthopaedic wards having more male patient admissions. Other authors also had similar findings. Tsakris et al, found 93.3 % of patients with MBL-PA were males and concluded that male gender was an independent high risk association [13].TABLE -2 In present study the diabetic patients were isolated MBL producing *P.aeruginosa* higher than the non diabetic patients due to poor blood glucose control which may contribute to increased mortality and morbidity since hyperglycemia is contributory to the development of infection and increased susceptibility to tissue injury.TABLE-3 The accurate identification and reporting of MBL-producing bacteria will aid infection control practitioners in preventing the spread of these multidrug-resistant isolates.

CONCLUSION

The early detection of MBL producing *P.aeruginosa* may help in appropriate antimicrobial therapy and avoid the development and dissemination of these multidrug resistance strains. To overcome the problem of emergence and the spread of multidrug resistant *P.aeruginosa* a combined intraction and cooperation between the microbiologists, clinicians and the infection control team is needed. We recommend a routine surveillance on antibiotic resistance in every clinical microbiology laboratory in order to aid infection control.

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