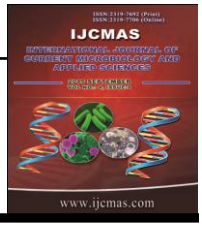
Int.J.Curr.Microbiol.App.Sci (2015) 4(9): 952-956

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 9 (2015) pp. 952-956**
http://www.ijcmas.com



Original Research Article

Prevalence of Metallo-Beta-Lactamase Producing *Pseudomonas aeruginosa* and its antibiogram in a tertiary care centre

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Keywords

Psedomonas aeruginosa, Metallo-β-lactamase, Imipenem resistance, CDT, DDST

A B S T R A C T

Metallo-β-lactamase (MBL) production is the important factor in causing carbapenem resistance in *Pseudomonas aeruginosa*. The study was done to find prevalence of MBL in *Pseudomonas aeruginosa*. 200 *Pseudomonas* isolates from various samples studied. Sample processing done following standard bacteriological standards. MBL were detected by combined disc test (CDT) & double disc synergy test (DDST). Wound swabs & tracheal aspirates constitute the major samples. Imipenem resistance found to be 30%. MBL producing strains were 25%. Combined disc method detected all MBL isolates while double disc synergy test only 65% of isolates. Most of pseudomonal infections are nosocomial in origin. CDT is best method for MBL detection. Cephalosporins are highly resistant. All strains are sensitive to Polymyxin-B & Colistin. Regular detection of MBL should be adopted for control of growing carbapenem resistance among *Pseudomonas aeruginosa*.

Introduction

Pseudomonas aeruginosa an opportunistic pathogen of humans is a gram negative, aerobic rod belonging to bacterial family Pseudomonadaceae. It causes life-threatening ventilator associated pneumonia, surgical site and urinary tract infections in patients from Intensive Care Units (Poirel *et al*, 2000).

Extended spectrum beta lactamases producing bacteria are typically resistant to penicillins, first and second generation cephalosporins as well as the third generation oxyiminocephalosporins (eg Ceftazidime, Ceftriaxone) and Monobactam (Aztreonam) (Rodrigues *et al.*, 2004).

Among these ESBL, Metallo-β-lactamases (MBLs) are zinc-dependent enzymes that have been gaining increasing attention because of their ability to hydrolyze nearly all known beta-lactams and of the lack of useful inhibitors. MBLs are enzymes belonging to Amblers Class B that can hydrolyze a wide variety of beta-lactam antibiotics including penicillins,

cephalosporins, and carbapenems, and requires divalent cation, usually zinc, as a cofactor for enzyme activity (Bush *et al* 1995).

Present study was conducted with an objective to find out the presence of MBL producing *P. aeruginosa* in multidrug resistant strains and to formulate effective antibiotic strategy and plan a proper hospital infection control strategy to prevent the spread of these strains.

Materials and Methods

In present study 200 isolates of *Pseudomonas aeruginosa* isolated from various clinical specimens in Basaweshwara hospital, a tertiary care centre in north-east Karnataka were studied for detection of metallobetalactamase production including their antibiogram.

All samples were collected under aseptic precautions and transported to the laboratory. Specimens were inoculated on to Blood agar and MacConkey's agar plate. A Brain Heart Infusion broth media was used for blood culture. Identification of *Pseudomonas aeruginosa* was done by Gram staining, culture characteristics and biochemical tests. The *Pseudomonas* isolates were subjected to antibiotic susceptibility testing by employing Kirby Bauer disc diffusion technique according to CLSI guidelines 2011. Methods used for detection of MBL detection were Imipenem(IMP)-EDTA combined disc test (CDT) as described by Yong *et al.* (2002) and Imipenem-EDTA double disc synergy test (DDST) as described by Lee *et al.* (2003).

Result and Discussion

The study constituted 200 isolates of *Pseudomonas aeruginosa* isolated from various samples. Age group of 31–70 years accounted for 78.5%. Of the total number of representative samples, 128 (64%) were males and 72 (36%) were females.

Wound swabs constituted majority of specimens accounting for 31% followed by tracheal aspirates 28%. BAL, urine and blood samples accounted for 11%, 8% and 8% respectively. 64 isolates (32%) were imipenem resistant which were further processed for detection of MBL. The majority of isolates (67%) of these were from wound swabs and tracheal aspirates. Imipenem resistant were 11% in BAL samples followed by 8% each in blood and urine. Of the 64 imipenem resistant pseudomonas isolates only 49 (76.5%) were MBL producers.

Table.1 Incidence of MBL producing imipenem resistant *Pseudomonas aeruginosa* (n=64) from clinical specimens

Clinical specimens	Isolates	Imipenem resistant	Percentage
Wound swab	62(31%)	27	42%
Tracheal aspirate	56(28%)	16	25%
Urine	31(15.5%)	5	8%
BAL	17(8.5%)	7	11%
Blood	14(7%)	5	8%
Sputum	08(4%)	2	3%
Others (ear, body fluids, cervical etc.)	12(6%)	2	3%
Total	200	64(32%)	

Table.2 Percentage of MBL and Non-MBL Producers among Imipenem Resistant Isolates

Organism	Total of imipenem resistant isolates	MBL producers	Non-MBL producer
Pseudomonas aeruginosa	64	49(76.5%	15(23.5%)

Table.3 Comparison of methods for detection of MBL in *P.aeruginosa*

Tests	Control (ATCC 27853) (*P. aeruginosa*)	No. of MBL positive (n=49)	Percentage positivity
CDT	Negative	49	100%
DDST	Negative	32	65%

Table.4 Resistance pattern of *Pseudomonas aeruginosa*

Antibiotic	No.	Percent
Cefotaxime	125	62.5
Ceftazidime	134	67.0
Netilmicin	111	55.5
Ciprofloxacin	107	53.5
Gentamicin	87	43.5
Tobramicin	76	38.0
Amikacin	82	41.0
Imipenem	64	32.0
Piperacillin + Tazobactum	76	38.0
Polymyxin-B	00	0.00
Colistin	00	0.00

The combined disc test (CDT) showed 49 isolates to be producing MBL, while double disc testing (DDST) detected in only 32(65%) isolates. About 67% of isolates of *Pseudomonas aeruginosa* were resistant to ceftazidime followed by cefotaxime (62.5%). Amongst aminoglycosides, least resistance was noted against tobramycin (38%), while higher resistance was noted for netilmicin (55.5%), gentamicin (43.5%) and amikacin (41%).

While resistance to imipenem and meropenem was noted in 32% cases, while piperacillin/ tazobactum accounts for 38% of resistance. The resistance pattern of *Pseudomonas* isolates is shown in table 4.

In the present study, an attempt was made to know the antibiotic profile of *Pseudomonas aeruginosa* with special concern to find MBL producing strains. Incidence is higher in males and in age group 31–70 years. Wound swabs constituted majority of specimens accounting for 31%, followed by tracheal aspirates 28%, urine and blood samples account for 8% each. These parameters indicate that most of these pseudomonas isolates are nosocomial in origin.

All Imipenem resistant isolates were screened for MBL production by combined disc test and double disc synergy test. MBL production in combined disc test is found in 49 isolates out of 64 Imipenem resistant isolates and in double disc synergy test

MBL production was seen in only 32 isolates. Prevalence of metallobetalactamase in Imipenem resistant isolates is 76% and is a major mechanism of carbapenem resistance. Prevalence of metallobetalactamase in *Pseudomonas aeruginosa* isolates is 25% in our hospital. Simit H. Kumar *et al.* (2012) also found the prevalence of MBLs to be 26.9% (Simit H. Kumar *et al.,* 2012).

The combined disc method (CDT) was found to be superior to DDST. This is in accordance with other published studies, which have found the combined disc method to be one of the most sensitive techniques for detecting MBL (Yan *et al.,* 2004). Combined disc test could be used as a convenient screening method in the clinical microbiology laboratories.

The multidrug resistance found to be more in MBL producers than non-MBL producers. Studies have shown higher prevalence of multidrug‐ and pandrug‐resistance among MBL‐producing strains, as compared to that in non‐MBL‐producing strains (Ranjan *et al.*, 2014). The cephalosporins were found to be more resistant (60–68%), while aminoglycosides showed varied resistance (40–55%). The piperacillin/tazobactum combination was found to be one of the good antibiotics against Pseudomonas in our study. In absence of therapeutic MBL inhibitors, polymyxins and colistin have shown to be effective in the treatment of multidrug resistant *P. aeruginosa* infections. However, they should not be used as monotherapy, but a combination therapy must be preferred.

Present study underlines the unique problem with MBLs, because of their broad spectrum and unrivalled drug resistance, creating a therapeutic challenge for clinicians and microbiologists. Hence we suggest the detection of ESBL and MBL in *Pseudomonas aeruginosa* should be a routine practice. To overcome the problem of emergence and the spread of multidrug resistant *P. aeruginosa* combined interaction and cooperation of microbiologists, clinicians and the infection control team is needed. We recommend the routine surveillance of antibiotic resistance in the hospital.

In the present study a total of 200 isolates of *P. aeruginosa* isolated from various specimens were studied. Majority of samples were from male patients. Pseudomonal infections were more common in age group 31 to 60 years. Wound swabs and tracheal aspirates were the most common samples. Combined disc test is simple, reliable and superior to double disc synergy test in detection of metallobetalactamases. Prevalence of metallobetalactamase in *Pseudomonas aeruginosa* isolates was 25% in our hospital. Metallobetalactamase in Imipenem resistant isolates was 76% and was a major mechanism of carbapenem resistance. Cephalosporins mainly ceftazidime and cefotaxime showed very high resistance, so should be used in combination therapy to prevent increasing resistance among cephalosporins group. Imipenem, meropenem, piperacillin/ tazobactum, amikacin and tobramycin reported less resistance among *Pseudomonas aeruginosa* isolates, so should be used judiciously and combination therapy should be preferred for preventing future resistance to these drugs. All MBL producing and non MBL producing Imipenem resistant *P. aeruginosa* isolates (100%) were sensitive to polymyxin B & colistin. To overcome the problem of emergence and the spread of multidrug resistant *P. aeruginosa*, the infection control practices, aggressive routine surveillance of

antibiotic resistance and the rational use of antibiotic policy in the hospital should be implemented.

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