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THE DETECTION OF METALLO BETA LACTAMASE PRODUCING PSEUDOMONAS AERUGINOSA IN A TERTIARY CARE HOSPITAL

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Abstract: Aim: The present study was undertaken to To detect presence of metallo beta lactamase producing *pseudomonas aeruginosa* in clinical isolates in our hospital. **Methods:** 50 non-repetitive clinical isolates consecutively isolated during the period of 6 months from August 2016 to February 2017 were included in the study. Various samples collected like urine, pus, blood, catheter tips, and body fluids. All isolates with gram negative bacilli, catalase and oxidase positive, polymyxin B sensitivity were included in this study. MBL detection was done by Imipenem-EDTA combined disk method (CDT). **Results:** Among 50 imipenem resistance *psuedomonas aeruginosa* isolates tested for metalobetalactamases 18 (36%) isolates were shown positive results and remaining 32 (64%) isolates were shown negative results. **Conclusion:** To conclude there are 36% of metallo beta-lactamase producing *Pseudomonas aeruginosa* prevalent in our area. Therefore, detection of these MBL producing *P. aeruginosa* is crucial for optimal treatment of critically ill patients and to prevent the spread of resistance.

Keywords: *Pseudomonas aeruginosa,* Metallo beta lactamase, Imipenem-EDTA combined disk method

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INTRODUCTION

Beta- lactam antibiotics are among the most often used antimicrobial agents and an increasing incidence of resistance to these drugs is a public health concern. These antibiotics act by inhibiting a set of transpeptidase enzymes (also called penicillin binding proteins or PBPS) that are essential for the synthesis of the peptidoglycan layer of the bacterial cell wall[1]. Beta lactam antibiotics are characterized by a four-membered beta lactam ring that serves as a substrate for the transpeptidase target enzymes.

There are hundreds of different beta lactams, and they are classified into groups based on structure[2,3]. Clinically important beta lactams include the penicillins, cephalosporins, carbapenems, and monobactams. There are several mechanisms by which bacteria acquire resistance to beta lactam antibiotics including efflux, reduced permeability, altered transpeptidases and inactivation by beta lactamases. The production of beta lactamase enzymes is the most common mechanism of bacterial resistance to beta lactam antibiotics.

Based on primary sequence homology, beta- lactamases have been grouped into four classes[4]. Classes A, C, and D are active-site serine enzymes that catalyse, via a serine –bound acyl intermediate, the hydrolysis of the beta lactam[5]. Class B enzymes require zinc for activity and catalysis does not proceed via a covalent intermediate but rather through direct attack of a hydroxide ion that is stabilized by the zinc in the active site[6,7].

Class B metallo beta lactamases have a broad substrate spectrum and can catalyse the hydrolysis of virtually all beta lactam antibiotics with the exception of monobactams. Also, In contrast to serine-based enzymes, MBLs are inactivated by metal chelators such as EDTA[8]. MBLs were initially discovered over forty years ago but were not initially considered a serious problem for antibiotic therapy because they were found chromosomally encoded and in non-pathogenic organisms[9,10]. This situation changed in the 1990s, however, with the spread of the IMP- and VIM-type metallo-beta-lactamses in gram-negative pathogens, including *Enterobacteriaceae,Pseudomonas aeruginosa*, and *Acinetobacter bumannii*[11,12].

Metallo beta lactamases producing pseudomonas aeruginosa stains are responsible for several nosocomial outbreaks in tertiary care centers across the world. It is well known that poor outcome occurs when patients with serious infections due to MBL producing organisms are treated with antibiotics to which the organism is completely resistant. Therefore, detection of these MBL producing *P.aeruginosa* is crucial for optimal treatment of critically ill patients and to prevent the spread of resistance. The present study was undertaken to determine *Pseudomonas aeruginosa* producing metallo beta lactamases in a tertiary care hospital, Tirupati.

MATERIALS AND METHODS:

Fifty non-repetitive clinical isolates consecutively isolated during the period of 6 months from August 2016 to January 2017 in Department of Microbiology, SVIMS, Tirupati were included in the study.

The following samples included were Urine, Blood, Catheter tips, Pus, Body fluids. Identification of *Pseudomonas aeruginosa* was done by-Gram's Staining, Colony morphologies on MacConkey agar, Motile, Oxidase test, Catalase, Polymyxin B sensitivity. The antibiotic sensitivity testing for *Pseudomonas aeruginosa* is carried out by Kirby Bauer's disk diffusion method.

In the present study 50 Imipenem resistant *pseudomonas aeruginosa* isolates are collected from various clinical samples based on criteria of Gran-negative bacilli which is oxidase positive, catalase positive, motile, and polymyxin-B sensitive and imipenem resistance on antibiogram. Various methods have been recommended for screening MBL. These include the modified Hodge test, double disc synergy test using imipenem and EDTA discs or ceftazidime and EDTA discs, EDTA impregnated imipenem discs and EDTA impregnated meropenem discs[13]. For MIC detection of imipenem, the E -test strip and microdilution (microtitre) plate method[14] is recommended.

Imipenem-EDTA combined disk method (CDT): A 0.5 M EDTA solution was prepared by dissolve 186.1 g of disodium EDTA in 1000 ml of distilled water and pH was adjusted to 8.0 by using NaoH. The mixture was then sterilized by autoclaving. The EDTA imipenem disks were prepared by adding a EDTA solution to 10-µg-imipenem disks to obtain a concentration of 750 µg. The disks were dried in an incubator and stored at 4ºC or at -20ºC in an air tight vial without desiccant. Test organisms were inoculated onto plates of MH agar (Opacity adjusted to 0.5 McFarland opacity standards). A 10-µg- imipenem discs and an imipenem plus 750 µg were placed on inoculated plates. The zone of inhibition around imipenem discs alone and those with EDTA was recorded and compared after 16-18 h incubation at 35°C. An increase in zone size of at least 7 mm around the imipenem-EDTA disc was recorded as a positive result. The difference in the resistance pattern of MBL-positive and MBL-negative was considered to be statistically significant if the P value was <0.05.

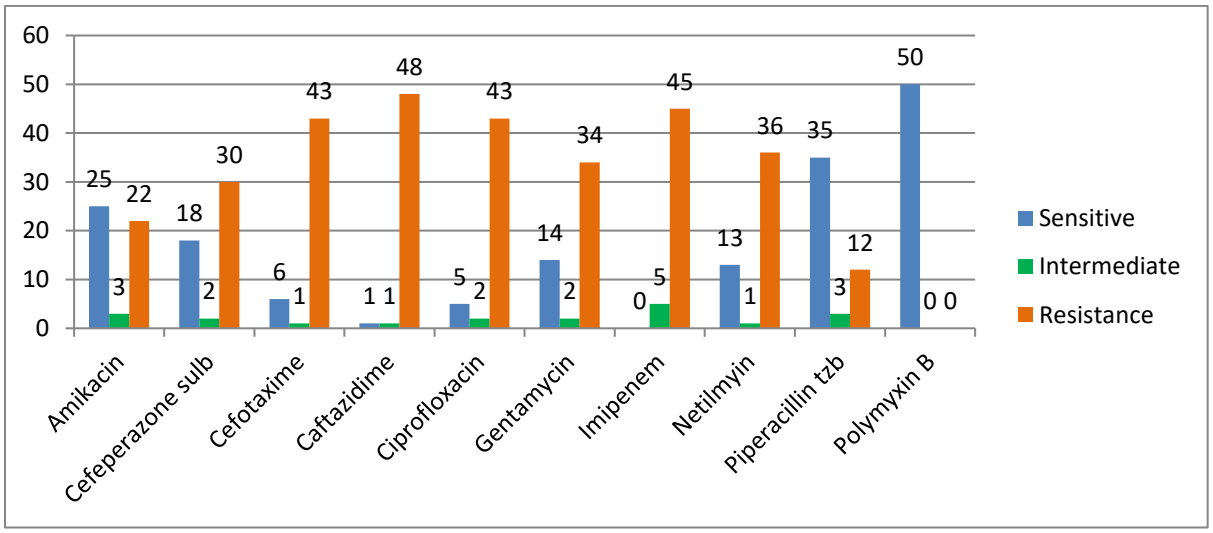
RESULTS:

The present study was conducted to detect metalobetalactamases producing *Pseudomonas aeruginosa* in a tertiary care hospital. Total 50 isolates were selected during Aug 2016 to Feb 2017 for the period of 6 months from various departments. All isolates are oxidase positive, catalase positive, gram negative bacilli, motile, imipenem resistant and polymixin B sensitive are included in this study. Out of 50 isolates, males were 27 and female were 23.

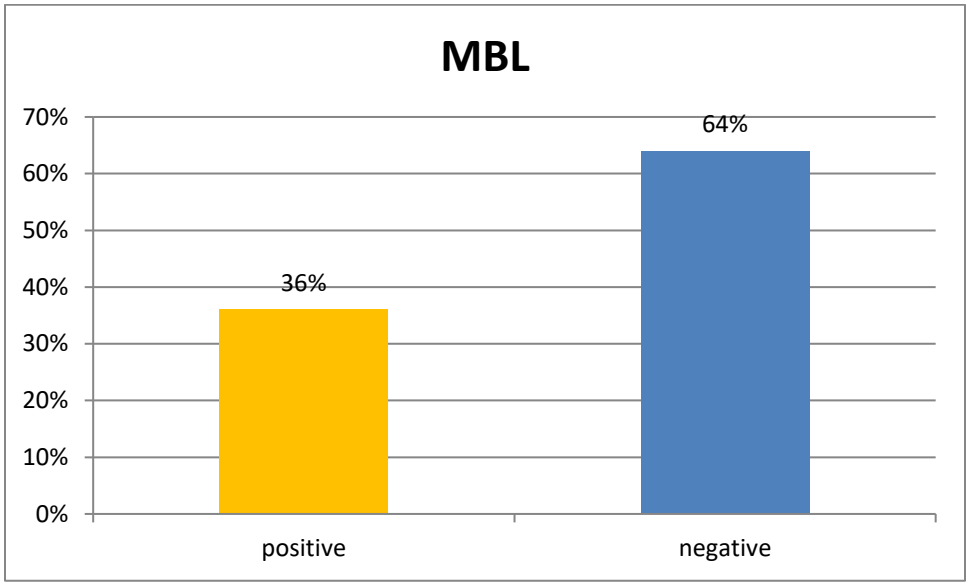
Among 50 samples urine samples were 25 (50%), sputum samples were 2 (4%), pus sample were 9 (18%), catheter tip sample were 9 (18%), body fluid samples were 1 (2%) and blood samples were 4 (8%).

All 50 pseudomonas isolates were tested for antibiotic sensitivity testing by using Kirby-bauer disk diffustion method. The various antibiotics include, amikacin, cefeperazone sulbactam, cefotaxime, ceftazidime, ciprofloxacin, gentamycin, imipenem, netilmycin, piperacillin tazobactam and polymyxin B. among 50 isolates 45 isolates shows resistance to imipenme remaining 5 isolates were intermediate sensitive.

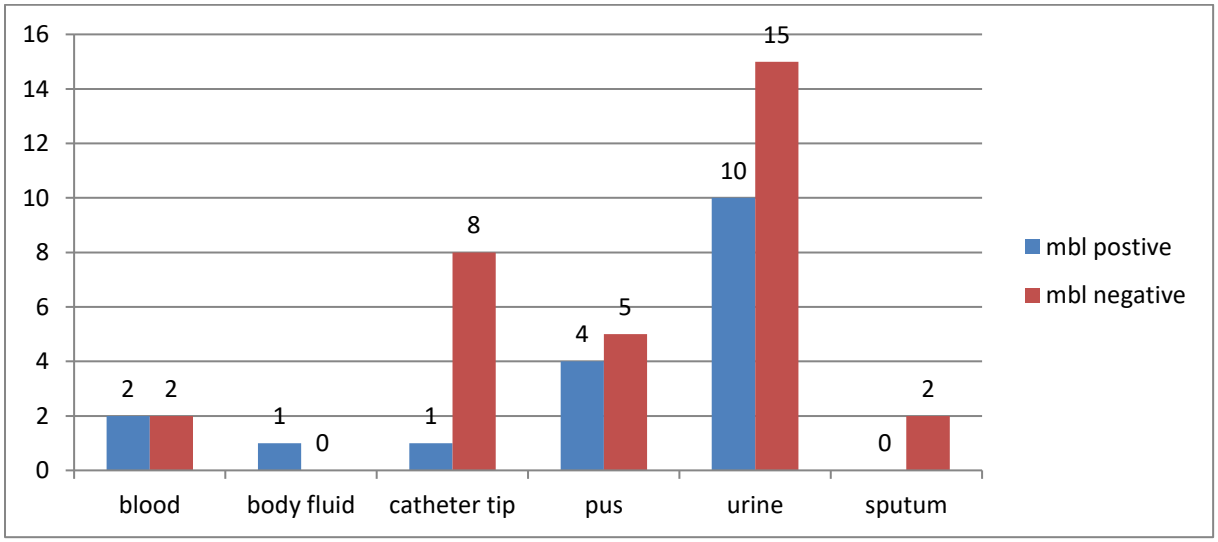
Highest sensitive noted to polymyxin B (100%) followed by piperacillin tazobactam (70%), amikacin (50%), cefeperazone sulbactam (36%), gentamycin (28%), netilmycin (26%), cefotaxime (12%), ciprofloxacin (10%) and ceftazidime (2%).



Among 50 imipenem resistance *Psuedomonas aeruginosa* isolates tested for metalobetalactamases by using imipenem-EDTA combined disk method (CDT). 18 (36%) isolates were shown positive results and remaining 32 (64%) isolates were shown negative results.



Among all 18 MBL positive isolates urine sample were 10, pus samples were 4, blood samples were 2, body fluids and catheter tip samples were 1, sputum samples were 0.



DISCUSSION:

In the present study a total of 50 pseudomonas isolates were investigated for the period of 6months from August 2016 to February 2017. The strains were collected from various clinical specimens including urine, sputum, blood, pus, catheters and body fluid samples from the patients of different inpatient and outpatient departments.

The present study was undertaken for detection of metallo β- lactamases production in *Pseudomanas aeruginosa*, due to the fact that pseudomonas are the leading cause of hospital acquired infection and opportunistic infections. The objective of the present study was to

determine the in clinical isolates of *Pseudomonas aeruginosa* species and prevalence metallo β-lactamase production among them.

Out of 50 isolates, males were 27 and female were 23. The highest percentage noted among males (54%) compare to females (46%). Among 50 isolates, urine samples were 25 (50%), sputum samples were 2 (4%), pus sample were 9 (18%), catheter tip sample were 9 (18%), body fluid samples were 1 (2%) and blood samples were 4 (8%). Highest percentage noted in urine samples (50%).

All 50 pseudomonas isolates were tested for antibiotic sensitivity testing by using Kirby-bauer disk diffusion method. Among 50 isolates, 45 isolates shows resistance to imipenem remaining 5 isolates were intermediate sensitive. Highest sensitive noted to polymyxin B (100%) followed by piperacillin tazobactam (70%), amikacin (50%), cefeperazone sulbactam (36%), gentamycin (28%), netilmycin (26%), cefotaxime (12%), ciprofloxacin (10%) and ceftazidime (2%). A similar study was conducted by Sunilkumar Biradar *et al*[107]in 2015 and Seema Bose in 2012 was reported all imipenem resistance strains are polymyxin B sensitive.

Among 50 imipenem resistance psuedomonas aeruginosa isolates tested for metalobetalactamases by using imipenem-EDTA combined disk method (CDT). 18 (36%) were detected as MBL isolates remaining 32 (64%) were negative. A similar study was conducted by P.Vasundhara Devi *et al*[16]in 2015 was reported 36% isolates were MBL positive.

CONCLUSION:

Beta- lactam antibiotics are among the most often used antimicrobial agents and an increasing incidence of resistance to these drugs is a public health concern. The increasing resistance is due to production of enzymes beta lactameses. Class B metallo beta lactamases have a broad substrate spectrum and can catalyse the hydrolysis of virtually all beta lactam antibiotics with the exception of monobactams. Metallo beta lactamases producing pseudomonas aeruginosa stains are responsible for several nosocomial outbreaks in tertiary care centers across the world. It is well known that poor outcome occurs when patients with serious infections due to MBL producing organisms are treated with antibiotics to which the organism is completely resistant. Therefore, detection of these MBL producing *P. aeruginosa* is crucial for optimal treatment of critically ill patients and to prevent the spread of resistance.

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