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Original research article

The Detection of Metallo Beta Lactamase Producing Pseudomonas Aeruginosa in A Tertiary Care Hospital

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ABSTRACT

Background: *Pseudomonas* species is responsible for 10% of nosocomial infections particularly in patients with burns and in an ICU environment. The most frequent mechanism of resistance to carbapenem, the most widely used medication to treat *Pseudomonas*, is the synthesis of metallo-β-lactamases (MBL). Both microbiologists and clinicians need to know the incidence of MBL-producing Pseudomonas in their area in order to develop an infection control plan for hospitals. The current study aimed to identify the incidence of MBL among clinical isolates of Pseudomonas species in a tertiary care hospital.

Methods: The samples include blood, urine, pus, body fluids, and catheter tips. The samples were cultivated on Blood agar, MacConkey's agar, and Thioglycolate broth, except for blood and urine samples. Brain Heart Infusion broth was used to culture blood. Blood and MacConkey's agar were used to culture urine. All culture plates were incubated overnight at 37°C. Identification of organisms was done by standard laboratory technique based on colony characteristics, Gram staining, and biochemical tests.

Results: All n=50 pseudomonas isolates were tested for antibiotic sensitivity testing by using the Kirby-Bauer disk diffusion method. Among the n=50 isolates n=45 isolates showed resistance to imipenem remaining n=5 isolates were intermediate sensitive. The highest sensitivity was noted to Polymyxin-B (100%) followed by Piperacillin+tazobactam (70%), Amikacin (50%), Cefoperazone+Sulbactam (36%), Gentamycin (28%), Netilmycin (26%), Cefotaxime (12%), Ciprofloxacin (10%) and Ceftazidime (2%). *Pseudomonas aeruginosa* isolates tested for metallo β-lactamases by using imipenem-EDTA combined disk method (CDT). N=18 (36%) isolates were shown positive results and the remaining n=32 (64%) isolates were shown negative results.

Conclusion: The development of MBL genes and their proliferation among bacterial pathogens are a topic of concern concerning the future of antimicrobial therapy since more and more MBL-producing *Pseudomonas aeruginosa* isolates are being identified as a major source of nosocomial infections. 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 the optimal treatment of critically ill patients and to prevent the spread of resistance.

Keywords: *Pseudomonas aeruginosa*, Metallo Beta Lactamase, Carbapenem, Imipenem

Introduction

One of the most frequent pathogens, *Pseudomonas aeruginosa*, causes serious infections that are challenging to treat, such as pneumonia and septicemia. It also causes nosocomial infection epidemics over the world. Isolates producing ESBL remain sensitive to carbapenems, after the discovery of carbapenemase-producing isolates which are resistant to all antibiotics except colistin, polymyxin B, and tigecycline acts as a precursor for an era of untreatable condition. [1] 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. [2] 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 based on structure. [3, 4] Clinically important beta-lactams include penicillin, cephalosporins, carbapenems, and monobactams. There are several mechanisms by which bacteria acquire resistance to beta-lactam antibiotics including efflux, reduced permeability, altered transpeptidase, 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. [5] Class A, C, and D are active-site serine enzymes that catalyze, via a serine-bound acyl intermediate, to hydrolysis of the beta-lactam. [6] Class B enzymes require zinc for activity and catalysis does not proceed via a covalent intermediate but rather through a direct attack of a hydroxide ion that is stabilized by the zinc in the active site. [7, 8] Class B metallo beta-lactamases have a broad substrate spectrum and can catalyze the hydrolysis of virtually all beta-lactam antibiotics except monobactams. Also, in contrast to serine-based enzymes, MBLs are inactivated by metal chelators such as EDTA. [9] 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. [10, 11] This situation changed in the 1990s, however, with the spread of the IMP- and VIM-type metallo beta-lactamases in gram-negative pathogens, including *Enterobacteriaceae, Pseudomonas aeruginosa*, and *Acinetobacter bumannii.* [12, 13] 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 the 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, in Tirupati.

Material and Methods

This cross-sectional study was conducted in the Department of Microbiology, SVIMS, Tirupati, Andhra Pradesh. Institutional Ethical approval was obtained for the study. Culture specimens were received from different wards, intensive care units, and outpatient departments. The samples include blood, urine, pus, body fluids, and catheter tips. The samples were cultivated on Blood agar, MacConkey's agar, and Thioglycolate broth, except for blood and urine samples. Brain Heart Infusion broth was used to culture blood. Blood and MacConkey's agar were used to culture urine. All culture plates were incubated overnight at 37°C. Identification of organisms was done by standard laboratory technique based on colony characteristics, Gram staining, and biochemical tests. [14] Pseudomonas aeruginosa-positive cultures were isolated and included in our investigation. They were then tested for antibiotic

sensitivity using commercially available discs (Hi-media, Mumbai) and the Modified Kirby Bauer disc diffusion technique. [15] The results were interpreted using antibiotics per CLSI standards. [16] Amikacin (30 µg), Gentamicin (10 µg), Netilmycin (30 µg), Cefoperazone + sulbactum (32 µg), Ceprofloxacin (1 µg), Ceftazidime (30 µg), Ceftriaxone salbactam (30 µg), Imipenem (10 µg), Piperacillin (100 µg) + tazobactum (100/10 µg), Polymyxin B (4 µg). The isolation of MBL-producing *P. aeruginosa* was suspected when the isolates were suspected to be imipenem-resistant. Three techniques were utilized to locate MBL.

Imipenem and EDTA combination disc test: A Mueller-Hinton agar plate was inoculated with an overnight broth culture of the test strain, with the opacity ultimately adjusted to 0.5 McFarland. Two 10 µg imipenem discs are dried, spaced 20 mm apart in the middle and one of the discs is given 10 µl of a 0.5 M EDTA solution. Following overnight incubation, an increase in the zone of inhibition of the imipenem/EDTA disc over the imipenem disc alone that was greater than 7 mm was deemed to be MBL positive. Disodium EDTA was dissolved in 1000 ml of distilled water to create a 0.5 M EDTA solution, and the pH was then brought down to 8.0 using NaOH. The mixture was then autoclaved to make it sterile.

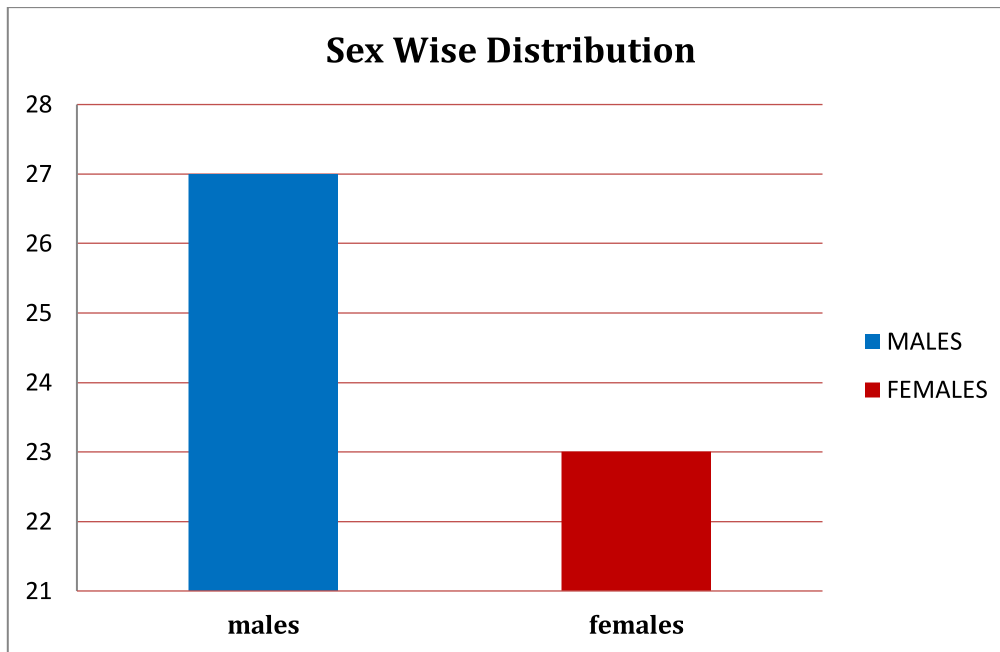
Double disc synergy test (DDST) using Imipenem and EDTA: As recommended by the CLSI, test organisms were inoculated on Mueller Hinton agar plates for the double disc synergy test (DDST) using imipenem and EDTA. A blank imipenem (10 µg) disc holding 10 µl of 0.5 M EDTA solution was positioned 20 mm from center to center apart from an imipenem. After overnight incubation, it was determined that the improvement of the zone of inhibition between the imipenem and the EDTA disc in comparison to the imipenem zone of inhibition was favorable.

MBL E-test: The E-test strip technique is used to detect imipenem's MIC. The Biomerieux E test MBL-strip has a double-sided seven-dilution imipenem gradient (IMP 4 to 256 µg/ml) at one end and imipenem (1 to 64 µg/ml) in conjunction with a fixed concentration of EDTA at the other end. After 24 hours of incubation, Mueller-Hinton agar plates were read after being inoculated with isolates corresponding to 0.5 McFarland standards. When the MIC of imipenem/imipenem-EDTA was more than 8, it was thought to be a sign that MBL was being produced.

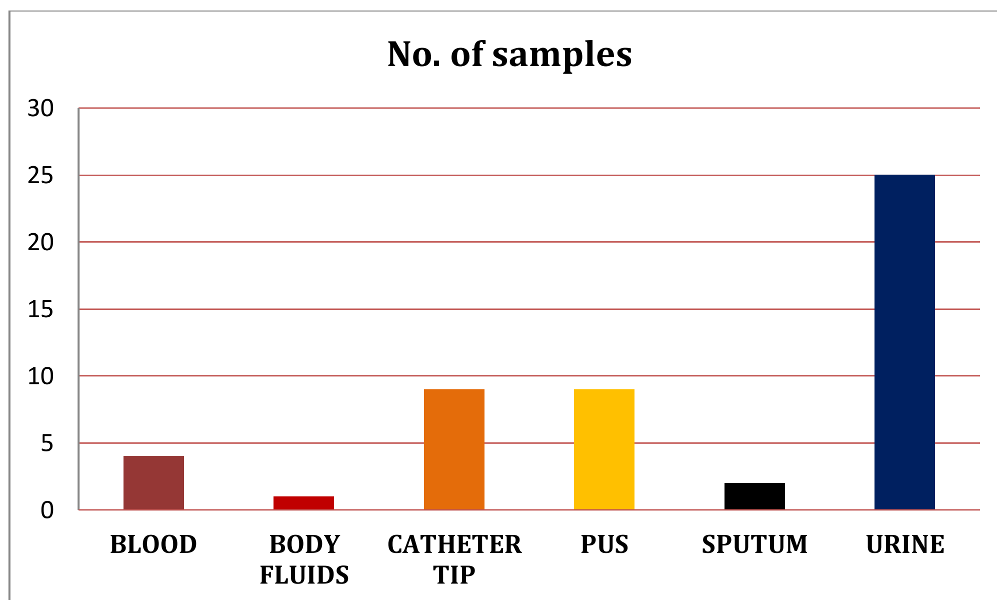
*Control***:** β lactamase negative *P. aeruginosa* ATCC 27853 strains were used as control.
Statistical analysis: All the data obtained was uploaded on an MS Excel spreadsheet and analyzed by IBM SPSS version 21.0 in Windows format (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). Continuous variables were represented by mean, standard deviations, and percentage. Categorical variables were represented by p values.

Results

The present study was conducted to detect metallo β-lactamase producing *Pseudomonas aeruginosa* in a tertiary care hospital, SVIMS Tirupati. A total of n=50 isolates selected from various departments were included in the study. All isolates are oxidase positive, catalase positive, gram-negative bacilli, motile, imipenem resistant, and polymyxin-B sensitive are included in this study. Out of n=50 isolates, males were n=27, and females were n=23. Males (54%) were higher than females (46%) depicted in graph 1. The range of age groups in the study was from 22 to 61 years. The mean age of the patients in the current study was 39.5 ± 8.5 years.



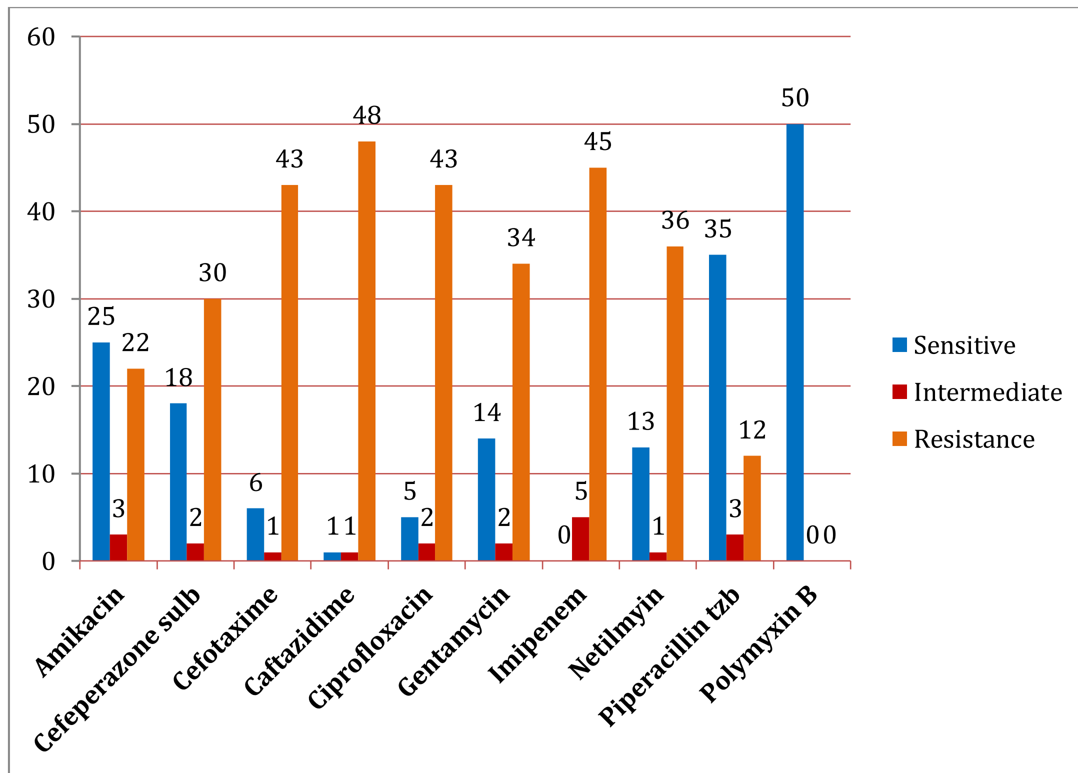
Graph 1: sex wise distribution of cases included in the study.



Graph 2: showing the samples collected from the cases in the study.

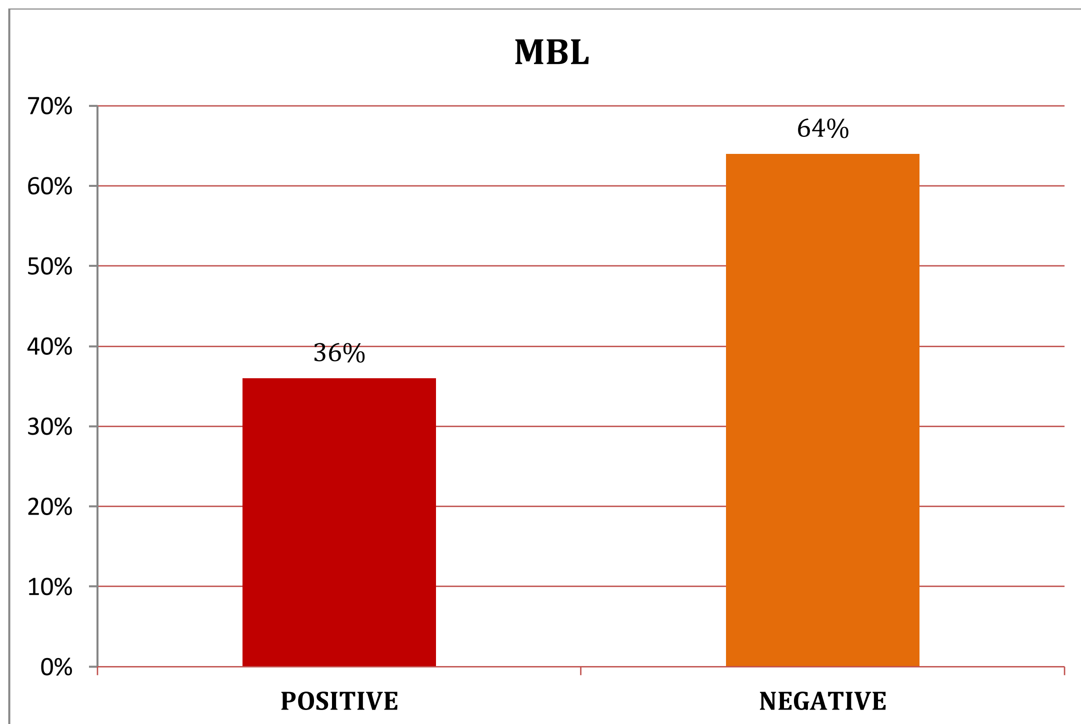
Among n=50 samples urine samples were n=25 (50%), sputum samples were n=2 (4%), pus samples were n=9 (18%), catheter tip samples were n=9 (18%), body fluid samples were n=1 (2%) and blood samples were n=4 (8%) depicted in graph 2.

All n=50 pseudomonas isolates were tested for antibiotic sensitivity testing by using the Kirby-Bauer disk diffusion method. The various antibiotics include amikacin, cefoperazone+sulbactam, cefotaxime, ceftazidime, ciprofloxacin, gentamycin, imipenem, netilmicin, piperacillin+tazobactam, and polymyxin-B. Among the n=50 isolates n=45 isolates showed resistance to imipenem remaining n=5 isolates were intermediate sensitive. The highest sensitivity was noted to Polymyxin-B (100%) followed by Piperacillin+tazobactam (70%), Amikacin (50%), Cefoperazone+Sulbactam (36%), Gentamycin (28%), Netilmycin (26%), Cefotaxime (12%), Ciprofloxacin (10%) and Ceftazidime (2%) depicted in graph 3.



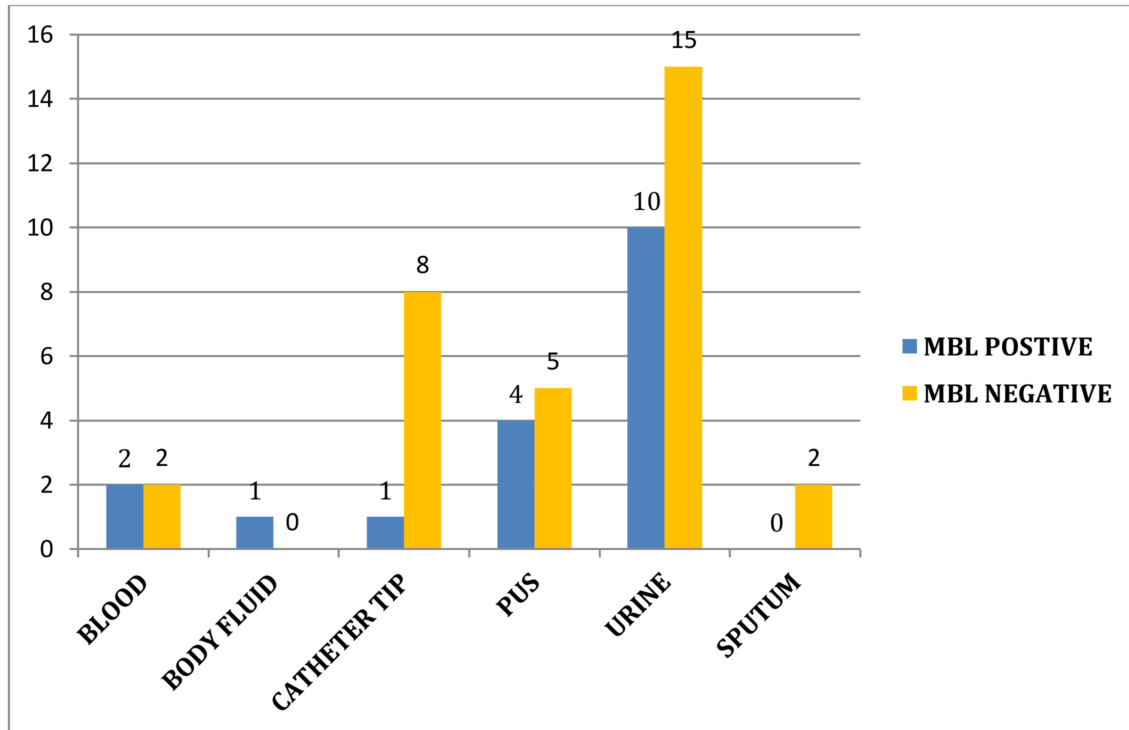
Graph 3: Antibiotic Sensitivity pattern recorded in the samples of the study.

Among n=50 imipenem resistance *Pseudomonas aeruginosa* isolates tested for metallo β-lactamases by using imipenem-EDTA combined disk method (CDT). N=18 (36%) isolates were shown positive results and the remaining n=32 (64%) isolates were shown negative results. (Graph 4).



Graph 4: Metallo beta lactamases cases in the study

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



Graph 5: Frequency of MBL in the samples of the study

Discussion

In the present study, a total of n=50 *Pseudomonas* isolates were investigated. 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 of SVIMS hospital. The present study was undertaken for the detection of metallo β-lactamases production in *Pseudomonas aeruginosa* because *Pseudomonas* is the leading cause of hospital-acquired infection and opportunistic infections. The objective of the present study was to determine the clinical isolates of *Pseudomonas aeruginosa* species and the prevalence of metallo β-lactamase production among them. Although multiple studies. [17, 18] have provided information on various elements of the epidemiology of nosocomial infections caused by Pseudomonas aeruginosa, not much tracking has been done to date to follow the resistance pattern in nosocomial infections. Given *Pseudomonas aeruginosa's* limited susceptibility to antibiotics, which has been supported by several study findings over the years [19], global drug resistance represents a significant medical catastrophe. [20]

Out of n=50 isolates, n=27(54%) isolates from males and n=23(46%) from female. Among n=50 samples, n=25(50%) samples were from urine samples, n=2(4%) were sputum samples, n=9(18%) were pus samples, n=9(18%) were catheter tip samples, n=1(2%) were body fluid samples, and n=4(8%) were blood samples, the highest percentage noted in urine samples (50%). The mean age of the patients in the current study was 45.5 ± 8.5 years. The mean age of the cases in the study is lesser compared to the mean age of cases reported by other similar studies. [21, 22] The higher proportion of samples in the study were from males because there is proportionately higher male opd and admissions as compared to females. Other authors have also found a similar observation. [21] Tsakris et al., [22] found that the male gender was an independent high-risk association and that 93.3% of patients with MBL-PA were men. In the

current study, we found all n=50 *Pseudomonas* isolates were tested for antibiotic sensitivity testing by using the Kirby-Bauer disk diffusion method. Among n=50 isolates, n=45 isolates were resistant to imipenem remaining 5 isolates were intermediate sensitive. The highest sensitivity was noted to polymyxin-B (100%) followed by piperacillin+tazobactam (70%), amikacin (50%), cefoperazone+sulbactam (36%), gentamycin (28%), netilmicin (26%), cefotaxime (12%), ciprofloxacin (10%) and ceftazidime (2%).

Different authors have provided descriptions of various resistance patterns. In contrast to VIM-2 type MBL-PA, which showed only 44% and 47% resistance to gentamicin and piperacillin-tazobactam, respectively, Tsakris et al., [22] observed 100% resistance to ceftazidime, cefepime, carbapenems, amikacin, netilmicin, and ciprofloxacin. According to a recent Indian study, the percentages of MBL-PA that were resistant to imipenem, gentamicin, ciprofloxacin, netilmicin, piperacillin, and amikacin, respectively, were 77.5%, 72.1%, 67.3%, 56.1%, and 55.1%. [23] A further investigation by De AS et al., [24] discovered complete resistance to all quinolones, beta-lactams, and aminoglycosides. 6 there are regional differences in susceptibility patterns, which reflect the prevalent antimicrobial practices in local hospitals. In this study among the n=50 *Pseudomonas aeruginosa* isolates tested for metallo-β-lactamases by using imipenem-EDTA combined disk method (CDT). N=18 (36%) isolates were shown positive results and the remaining n=32 (64%) isolates were shown negative results. The prevalence of MBL production among P. aeruginosa in our hospital was 36%. Similar observations were made by P. Vasundhara Devi et al., [25] in 2015 was reported 36% of isolates were MBL positive. This study suggests that the prevalence of *P. aeruginosa* is increasing and the gene responsible for MBL synthesis is carried on mobile genetic elements in the future, which might lead to a quick spread to sensitive bacteria in the hospital and alter this scenario. This highlights the need of identifying isolates that produce Metallo beta-lactamases, practice stringent infection control, and using carbapenems and other broad-spectrum beta-lactamases only when necessary in clinical settings. Regular MBL identification will guarantee the best patient care and prompt implementation of the necessary infection control measures.

Conclusion

The current investigation aimed to determine the prevalence of MBL-producing *P. aeruginosa* and potential therapeutic options. The development of MBL genes and their proliferation among bacterial pathogens are a topic of concern concerning the future of antimicrobial therapy since more and more MBL-producing *Pseudomonas aeruginosa* isolates are being identified as a major source of nosocomial infections. 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 the optimal treatment of critically ill patients and to prevent the spread of resistance.

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