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Detection of Metallo-Beta-Lactamase (MBL) Producing *Pseudomonas aeruginosa* in a Tertiary Care Hospital, Udaipur, Rajasthan

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ABSTRACT

Introduction: *P. aeruginosa* is a prevalent pathogenic agent in neonatal critical care units due to its widespread distribution, strong preference for wet environments, and capacity to thrive in a variety of settings. Predisposing factor with considerable morbidity and fatality rates in *Pseudomonas* septicemia are invasive diagnostic and therapeutic procedures, and indiscriminate antibiotic usage *Pseudomonas aeruginosa* has been found to cause a mixture of infections in clinical practice: like chronic Cystic fibrosis (CF) lung infection, septicemia in burn patients, surgical wound infection, urinary tract infection, corneal ulceration and Infrequently, *P. aeruginosa* can colonize human body sites, with a partiality for moist areas, such as the perineum, axilla, ear, nasal mucosa, throat, as well as stools.

Aims & objectives: Isolation and identification of *Pseudomonas species*. To find out prevalence of *Pseudomonas aeruginosa* from various clinical samples. To detect Metallo beta lactamase in Pseudomonas species isolated from various clinical samples.

Materials and methods: Various clinical samples like Urine, Endotracheal, Tracheostomy, Blood, Pus, Sputum, and CSF were collected by aseptic technique in sterile container except blood which is collected in blood culture bottle. Antimicrobial susceptibility testing will be done on Mueller Hinton agar (MHA) according to CLSI guidelines for Kirby Bauer disc diffusion test. After 24 hours of incubation zone of inhibition were measured. Phenotypic metallo-beta-lactamase also detected as per standard guidelines.

Results and Observation: In our research study we have analyzed 1000 various clinical samples i.e Urine, ET, TT Blood, Pus, Sputum, and CSF and isolate 400 samples of pseudomonas species. Out of 400 *Pseudomonas species* 300 were identified as *Pseudomonas aeruginosa.* The prevalence of *Pseudomonas aeruginosa* was 30 percent. Detection of Metalloid-β-lactamases (MBLs) producing *Pseudomonas aeruginosa.* In our study we have noted that maximum resistance was found in Imipenem 112 (37.33%). Out of 112 imipinem *resistant Pseudomonas aeruginosa*, 40 (35.71%) were detected as a MBL producer. Phonotypical detection of Metallo-β-lactamases.

Discussion and Conclusion: The emergence of carbapenem resistance reflects a threat limiting treatment choices and suggests the need for on-going epidemiological and antimicrobial susceptibility studies and longitudinal surveillance of antibiotic prescription. Increase in antibacterial resistance in P. aeruginosa is a cause of concern. So, continuous monitoring of bacterial resistance trends should be done and therapy should be based on antibacterial susceptibility results...

Keywords: *Pseudomonas aeruginosa, antimicrobial resistance, Carbapenem, Metallo-β-Lactamase.*

1. INTRODUCTION

The frequency of colonization in healthy individuals is usually low, higher colonization rates can be encountered following

hospitalization, particularly among patients treated with broad- spectrum antibiotics [1]. Usually, for an infection to occur, there should be some disruption of the physical barriers (skin or mucous membrane), or by passing of invasive devices or an underlying dysfunction of the immune defense mechanisms [2].

P. aeruginosa is mostly a nosocomial pathogen. Infections associated with this bacterium are nosocomial respiratory tract infections which integrated ventilator associated pneumonia (VAP), dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a mixture of systemic infections, particularly in immune suppressed patients, or patients with severe burns and cancer [3]. Community acquired infections caused by *P. aeruginosa* are uncommnon. The most common ones are: urinary tract infections, otitis externa, folliculitis acquired in swimming pools, keratitis due to wearing contact lenses [4]. The mucoid phenotype of *P. aeruginosa* frequently colonises and infects patients with cystic fibrosis of the lung causing damage of the lung tissue and decreased pulmonary function [5]. *P. aeruginosa* have an abundance of virulence factors, including flagella, pili, lipopolysaccharides, alginate, alkaline protease, phospholipase C, and exotoxin A, quorum sensing mechanisms, type II secretion system, pyocianin, pyoverdin [6]. It also produces a number of toxic proteins which not only cause extensive tissue damage, but also interfere with the human immune system's defense mechanisms [7].,

Bacteremia

One of the top 5 causes of nosocomial bacteremia, which can result in sepsis, is *P. aeruginosa*. In the 1960s and early 1970s, the sole therapies for *P. aeruginosa* bacteremia were aminoglycosides and polymyxins, but it was later shown that these medications were useless for these infections. When mortality was used as the end point, it was discovered that mortality rates were higher than 50%, reaching rates of up to 70% in febrile neutropenic patients. *P. aeruginosa* penetrates the epithelial and endothelial tissue barriers to enter the bloodstream when it spreads from a local infection [8].

Patients with *P. aeruginosa* bacteremia have been found to have crude death rates of more than 50%. As a result, this clinical entity has been widely feared, and several antibiotics have been used to try to manage it. According to recent papers, attributable death rates range from 28 to 44 percent, with the exact number dependent on the severity of the underlying condition and the effectiveness of therapy [9]. The patient with *P. aeruginosa* bacteremia used to typically be neutropenic or have burn damage. Today, though, bacteremic *P. aeruginosa* infections are present in just a small proportion of individuals in these categories. Instead, ICU patients with *P. aeruginosa* bacteremia are most commonly affected [10].

Aims:

- Isolation and identification of Pseudomonas species
- To find out prevalence of Pseudomonas aeruginosa from various clinical samples.
- To detect Metallo-beta-lactamase in Pseudomonas species isolated from various clinical samples.

Objectives:

- Present study will help to know the prevalence of Pseudomonas aeruginosa species from various clinical samples and their antimicrobial resistant pattern
- Detection and close monitoring of drug resistant strain will help in management of infections caused by Pseudomonas aeruginosa species and formulation of antibiotic policy in hospital and appropriate infection control measures to reduce the incidence of antimicrobial drug resistance.

2. MATERIALS AND METHODS

This prospective study was carried out from May 2020 to December 2022 in department of Microbiology in Pacific Medical College and Hospital Udaipur, Rajasthan. It was done on various clinical samples i.e Urine, Endotracheal, Tracheostomy, Blood, Pus, Sputum, and CSF were collected from IPD and OPD patients. The collected samples were inoculated onto Nutrient agar, Blood agar and MacConkey agar plates. All plates were incubated aerobically at 37°C and observed for microbial growth at 24 and 48 hours. Identifications were done on the basis of phenotypically and biochemical characterizations [11].

Antimicrobial Susceptibility Testing

Touch four or five isolated colonies of the organism to be examined with a sterile inoculating loop. The organism was suspended in an appropriate broth medium and incubated for 4-6 hours at 35-37°c., the inoculums were swabbed over the dried surface of the Muller Hinton agar plate and left to dry at room temperature, as per procedure. Antibiotic discs were applied with proper pressure using sterile fine pointed forceps. The plates were incubated aerobically for 10 to 24 hours at 37°C.

Antimicrobial susceptibility testing will be done on Mueller Hinton agar (MHA) according to CLSI 2022 guidelines for

Kirby Bauer disc diffusion test. After 24 hours of incubation zone of inhibition were measured [12].

Statistical criteria to select the minimum sample size: Calculation:

$n = Z2 \times P \times Q/E2$

Based on statistical analysis the minimum sample size required in our study is 279 and we have taken total sample size of 1000 and out of these samples we have analyzed total 300 samples of Pseudomonas aeruginosa which are quite relevant to our study.

Statistical analysis: The data was coded and entered into a database on an Excel spreadsheet and analyzed using Statistical Package for the Social Sciences (SPSS) version 23.0.

The descriptive analysis was performed to calculate the frequency and categorical variables were expressed as proportions (%). All statistical analysis was done with statistical significance set at≤0.05.

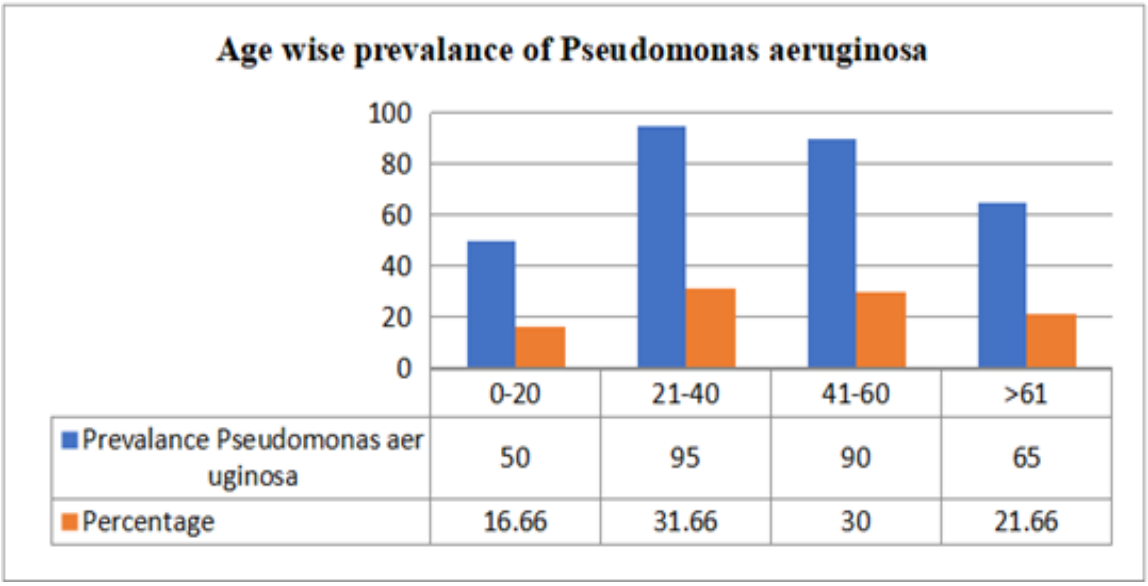
Combined Disc Synergy Test (CDST) [13]. The IMP-EDTA combined disk test was performed as described by yong et al. [14]. Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI. Two 10ug Imipenem disks (Hi Media) were placed on the plate with distance of 10 mm edge to edge between plane IMP and IMP-EDTA combined disc. 10 ul of EDTA solution was added to one of them to obtain the desired concentration (750 ug). The inhibition zones of the Imipenem and -EDTA disks were compared after 16-18 hours of aerobic incubation at 35C. Imipenem If the increase in inhibition zone with the Imipenem and EDTA disc was >7 mm than the Imipenem disc alone, it was considered as MBL positive [15].

3. RESULTS AND OBSERVATIONS

In our research study we have analyzed 1000 various clinical samples i.e Urine, ET, TT Blood, Pus, Sputum, and CSF and isolate 400 samples of pseudomonas species. Out of 400 Pseudomonas species 300 were identified as Pseudomonas aeruginosa the basis of morphological and biochemical estimation. These 300 samples of Pseudomonas aeruginosa were further processed to study the antimicrobial resistance patterns and detect metallo beta lactamase.

maximum number of *Pseudomonas aeruginosa* was found in indoor male patients i.e 56% followed by 52% in OPD male patients while minimum *Pseudomonas aeruginosa* was found in female patients in IPD i.e 44% followed by 48% in OPD. Study clearly revealed that male patients are more prone to *Pseudomonas aeruginosa* infection as compared to female patients.

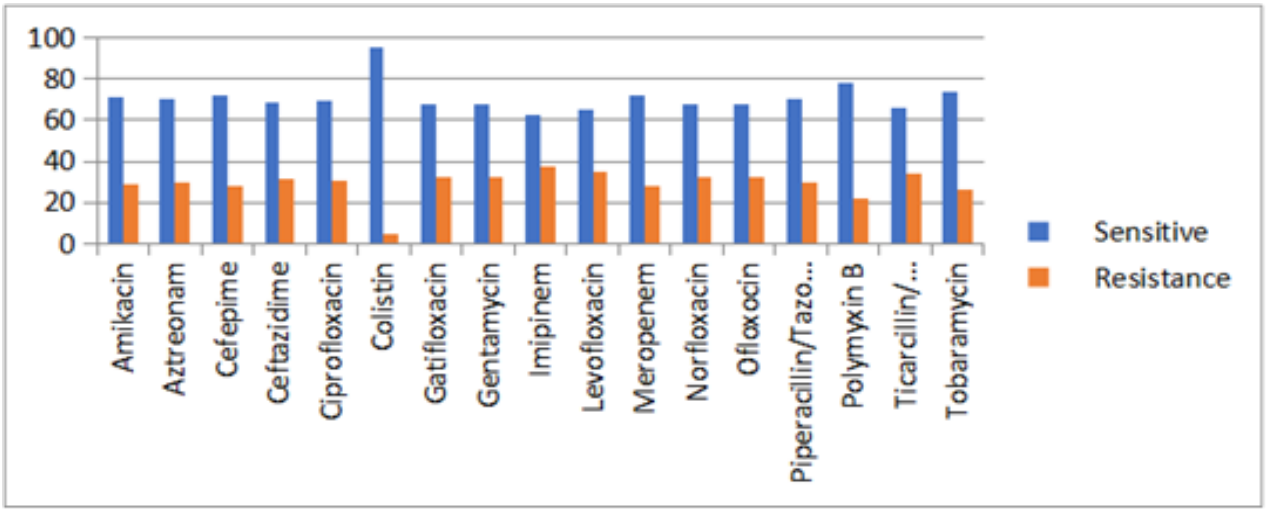
Table 1: Age wise distribution of patients with *Pseudomonas aeruginosa* infection



	0-20	21-40	41-60	>61
Prevalance Pseudomonas aeruginosa	50	95	90	65
Percentage	16.66	31.66	30	21.66

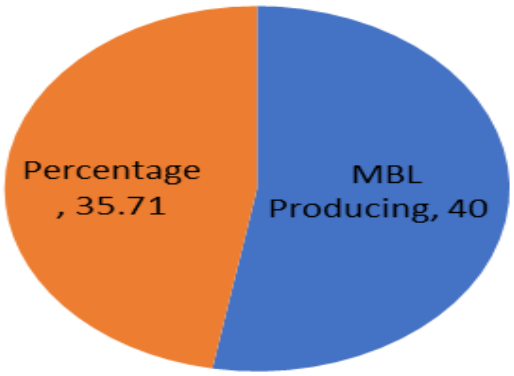
Our findings clearly shown that age group is more prone to pseudomonas infection i.e. 31.66% followed by followed by 41-60, >61 and 0-20 i.e 30%, 21.66% and 16.66%.

Table 2: Antibiogram of *Pseudomonas aeruginosa* from various clinical samples



Antibiogram of *Pseudomonas aeruginosa* clearly revealed that Colistin shown maximum sensitivity i.e (95%) followed by Polymixin B (78.33%), Tobramycin (73.33%), Cefepime and Meropenem (71.66%). Antibiotic Amikacin have shown sensitivity i.e (71.33) respectively. Piperacillin/Tazobactam and Aztreonam have shown similar sensitivity i.e 70% against *Pseudomonas aeruginosa* infection. Ciprofloxacin, Ceftazidim and Gentamycin have shown 69.66%, 68.33% and 68% sensitivity. Antibiotic Norfloxacin and Gatifloxacin have shown similar sensitivity i.e 67.66% followed by Oflaxacin, Ticarcillin/Clavulanicacid and Levofloxacin i.e 67.33%, 66% and 65% respectively. Our study clearly indicates that Imipenem have shown minimum sensitivity I.e 62.66%.

Incidence of MBL producing *Pseudomonas aeruginosa*



Out of 300 *Pseudomonas aeruginoisa* isolates 112 (37.33%) were shown imipenem resistance, and out of thses 112 imipenem resistance *Pseudomonas aeruginoisa* 40 (35.71%) were identified as a MBL producer.

4. DISCUSSION

In our study, in 2023 at Udaipur, all MBL positive strains were resistant to Aztreonam which means 0% sensitivity which is comparable with the studies done by Rakesh et al,,in 2014 at jaipur 0% of MBL producing isolates were sensitive to Cefoperazone/ Sulbactam. Higher sensitivity of MBL producing strains was found by is Fang D, Xi-wei X, Wen-qi S, et al. (12.5% sensitivity) in 2008 at Beijing (China). The study showed 15 % of MBL producing isolates to be sensitive to Ciprofloxacin which is comparable to the study by Rakesh et al. the study showed 12 % of MBL producing isolates to be sensitive to Ciprofloxacin which is comparable to the study by others. (10% Sensitivity) in 2014 at New Delhi (India) [16].

Higher sensitivity Saha R, Jain S and Kaur IR (12% Sensitivity) in 2010 at New Delhi (India). Higher sensitivity was reported by Fang D, Xi-wei X, Wen-qi i S, et al. (100% sensitivity), in 2008 at Beijing (China). Lower sensitivity was shown by Varaiya A, Kulkarni N, Kulkani M, et al. 2% in 2008 at Mumbai (Maharastra) [17]and Lee K, Chong Y, Shin HB, et al. [18] 4% in 2001 at (China). In the present study, Gentamicin showed 10% sensitivity among MBL producing isolates. Higher sensitivity was shown by Fang D, al. Xi-wei X, Wen-qi S, et (17.9% sensitivity) in 2008 at Beijing (China). 0% Franco M

MRG, Caiaffa-Filho H H, Burattini MN, et al. at [19] Sao Paulo (Brazil) during sensitivity was reported by in our study, which is similar to Saha Iain S and Kaur IR in 2010 at New Delhi (India) [20], Attal RO, Basak S, Mallick SK, Bose S. et al. in 2010 [21] at JNMC Wardha (Maharashtra), Franco MRG, Caiaffa-Filho HH. Burattini MN, et al. Sao Paulo (Brazil) during 2010 and Lee K, Chong Y, Shin HB, et al. in 2001 at at Seoul (Korea) [22,23].

5. CONCLUSION

β-lactam antibiotics are a key tool in the treatment of *Pseudomonas aeruginosa* infections and will remain so for the foreseeable future. However, the occurrence of resistant isolates of this major and problematic pathogen, complicating treatment, is a major concern. Clinical isolates that have acquired carbapenemases are a serious problem as carbapenems are one of the last lines of defence against *Pseudomonas aeruginosa*. The importance of β-lactams in treating *Pseudomonas aeruginosa* infections, and the widespread occurrence of antibiotic-resistant bacteria, has led to a large amount of research into the mechanism of β-lactam action and bacterial resistance

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