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RESEARCH ARTICLE
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ANTIBIOTIC RESISTANCE PATTERNS OF MBL-PRODUCING *PSEUDOMONAS AERUGINOSA* ISOLATED FROM HOSPITAL SETTINGS IN QUETTA, BALOCHISTAN

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ABSTRACT

Objective: This study aimed to examine the variety of clinical specimens collected from admitted patients, perform isolation and identification of metallo-β-lactamase (MBL)-producing *Pseudomonas aeruginosa* and their antibiotic resistance patterns.

Methods: The cross-sectional study was conducted at Microbiology Laboratory of BMCH Quetta from June 2022 to June 2023. A total of 93 strain of *P. aeruginosa* were isolated from pus, urine, wounds, sputum and pleural fluid. All bacterial isolates were identified through established standard methods, and their antibiotic susceptibility was assessed using the Kirby-Bauer disc diffusion technique. This testing adhered strictly to the guidelines set forth by the Clinical and Laboratory Standards Institute (CLSI). Furthermore, imipenem-resistant strains were assessed for metallo-beta-lactamase (MBL) activity using the double-disk synergy assay.

Results: Among the 93 tested *P. aeruginosa* isolates, 24 exhibited resistances to Imipenem (IMP), with 18 (75%) of them being metallo-β-lactamase (MBL) producers. All MBL-positive isolates (100%) showed resistance to Cefotaxime, Ceftazidime, and Imipenem. Additionally, MBL-positive strains exhibited varying resistance patterns: Polymyxin B demonstrated a sensitivity of 6.67%, Piperacillin-tazobactam showed 66.66%, Amikacin exhibited 83.33%, Tobramycin reached 83.33%, and Ciprofloxacin displayed a sensitivity of 72.22%. In terms of ward distribution, the majority of samples, 43 (12.53%) were from the surgical unit, followed by 34 (9.91%) from medical wards, and 16 (4.66%) from the ICU.

Conclusion: Timely identification of *P. aeruginosa* that produces MBLs helps healthcare professionals choose appropriate antibiotics, implement infection control, and adapt treatment strategies to improve patient outcomes while controlling antibiotic resistance.

Keywords: *P. aeruginosa*, MBL antibiotics, Balochistan.

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative, aerobic, non-fermentative, and non-spore-forming opportunistic pathogen, particularly in hospital environments.[1] It affecting around 9–10% of hospital-acquired infections and is a prevalent cause of such illnesses.[2] This is frequently associated with septicemia, pneumonia, sever urinary tract infections, surgical infections and other healthcare

related conditions.[3] Individuals with impaired immune systems are more vulnerable to infections, and their mortality rates might be from 30 to 50 percent. Patients suffering illnesses like AIDS, organ transplant recipients, and those enduring chemotherapy are examples of individuals. Furthermore, patients who stay in the hospital for longer than a week are at a higher risk of *P. aeruginosa* infections. This is often caused by factors such as the use of invasive medical equipment, prolonged antibiotic therapy, and exposure to hospital environments where the bacteria may be present.[4]

Bacteria, being highly adaptable microorganisms, have developed an array of sophisticated mechanisms to minimize the impact of antibiotics. Among the various strategies employed by bacteria against antibiotics include reduced outer membrane permeability, target modification, inactivation by β-lactamases, and the use of efflux pumps among the various strategies they employ. Since MBL-producing *P. aeruginosa* is considered one of the most concerning resistance mechanisms, it poses a growing threat and a serious public health concern. Treating infections caused by *P. aeruginosa* is particularly challenging because it produces β-lactamases, which hydrolyze a wide range of β-lactam antibiotics, including carbapenems—often used as last-resort options. This ability significantly compromises the efficacy of these critical antibiotics, leading to an alarming increase in treatment failures and a heightened risk of difficult-to-manage, widespread infections.[5]

A number of phenotypic screening methods for β-lactamases production have been introduced all of which based on the capacity of metal chelators like EDTA and thiols to deactivate MBLs. In these tests metal chelators, EDTA and thiols are used in combination with beta-lactam antibiotics CAZ and IMP in different settings.[6,7] Intrinsic resistance of *P. aeruginosa* is a combination of decreased permeability of outer membrane and secondary resistance mechanisms which involve energy driven efflux and β-lactamases.[8]

Finding efficient methods for identifying metallo-beta-lactamase (MBL) producers is essential, especially in view of the increasing be concerned across antibiotic resistance, especially in context of *P. aeruginosa* infections. The Combined Disc Synergy Test (CDST), one of the several methods that are available, has shown potential because of its ease of application, cost-effectiveness, and reliability in differentiating isolates that produce MBL from those that non producer MBL. This study explores the importance of MBL producers in *P. aeruginosa* isolates to combat antimicrobial resistance, highlighting the effectiveness of the CDST approach.

This study will help assess the prevalence of MBL-secreting *P. aeruginosa,* characterize the phenotypic antibiotic resistance patterns among clinical strains, and identify risk factors for treatment failure and mortality.

METHODS

This study was conducted for characterization and identification of metallo-beta-lactamases acquired by *P. aeruginosa* in the Microbiology laboratory of BMCH Quetta from June 2022 to June 2023.The sample size was determined based on a significance level of 5%, a confidence level of 95%, and a test power of 80%.

Sample collection:

Total 343 samples were collected from tertiary care hospitals Quetta, under aseptically conditions and processed according to standard guidelines.[9] This study included a diverse patient population, which included voluntary participation, and ensured that the consent process followed ethical guidelines. The excluded patients were those who had taken broad-spectrum antibiotics during the 48 hours before the sample was collected. Each sample was taken with sterile swab and after labeling immersed in the container and directly transported to the BMCH laboratory for comprehensive and detailed analysis. Out of the total samples, 93 strains of *P. aeruginosa* were successfully isolated from different clinical specimens, such as urine, wound exudates, pus, pleural fluid, and sputum samples. To maintain safety, 70% ethanol was used to routinely disinfect the

working area, and all gloves, microscope slides, masks, and cultures were autoclaved prior to disposal.

Isolation and identification of *P. aeruginosa*:
The suspected samples were streaked onto Blood agar, MacConkey agar, and Citramide agar, a selective medium. Following a 24-hour incubation at 37°C, the resulting colonies underwent Gram staining. The isolates were then identified using the analytical profile index (API) 20E and API 20NE systems (bioMérieux, France), following the manufacturer's instructions.

Antibiotic susceptibility testing: The resistance profile of *P. aeruginosa* isolates was assessed using Mueller-Hinton agar (MHA) against a various antimicrobial agent calibrated to a 0.5 McFarland standards and tested with commercially available discs (Oxoid, UK) by the Agar-based disk diffusion assay.[10]
The antibiotic susceptibility was evaluated using various commercially obtained antibiotics (Oxoid, UK). The diameter of the inhibition zones was carefully measured and precisely interpreted in strict accordance with the latest CLSI guidelines.[11]

Identification of MBL Production:
Imipenem-EDTA Combined Disc Test (CDST):
Those isolates showed imipenem resistance; it was suspected that MBL produced *P. aeruginosa*. To assess MBL production in the imipenem-resistant isolates, the IMP-EDTA combined disk test was performed according to the guidelines defined by CLSI.[12]The selected organisms were inoculated onto Mueller-Hinton agar plates calibrated to a 0.5 McFarland standard. After allowing the agar plates to dry for ten minutes, two imipenem discs (each containing 10 µg) were placed on the surface approximately 20 mm apart. One disc was impregnated with 0.5 µl of M EDTA, while the other was left plain. Afterward, the plates were incubated overnight at 37°C. Using the combined Disc Test, an increase in the inhibition zone of ≥ 7 mm with the Imipenem and EDTA disc compared to the Imipenem disc alone was considered indicative of an MBL-positive result as shown in Figure 1.
The statistical analysis included frequency analysis of the MBL positive strain and antibiotic susceptibility test. Furthermore, the paper provides visual representations, including pie charts and bar charts, to enhance comprehension. All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS). Ethical approval for the research was obtained from the Ethics Review Committee of the BMCH Quetta (Approval No. Supdt: /BMCH /3023/2023) and the research was conducted in compliance with the Helsinki Declaration.

RESULTS
Total 343 samples out of which 93 (27.11%) isolates of *Pseudomonas aeruginosa* were obtained from different specimen further out of 93 (27.11%) isolates 24 (6.99%) were imipenem-resistant (IMR) *P. aeruginosa* isolates as shown in Figure 2.
The results stratified by age indicated that the percentage of positive isolates was higher at 11.66% among peoples aged 21 to 40, than in other age groups, as illustrated in Figure 3.
Furthermore, notable number of isolates was found in male individuals, totaling 54 (15.74%), which is higher than that observed in female individuals 39 (11.37 %).
According to ward distribution, highest number of samples 43 (12.53%) were from surgical wards followed by 34 (9.91%) from medical wards and 16 (4.66%) were from ICU unit as shown in Figure 4.

In the present study, among all isolates examined, 65 (69.89%) exhibited resistance to Cefotaxime, while 63 (67.74%) showed resistance to Ceftazidime. Notably, resistance to Imipenem was observed in 24 (25.80%) of the isolates, as illustrated in Figure 5.

Of the 24 IMP resistance *P. aeruginosa* isolates, of which 18 isolates were MBL producers Furthermore, all 18 metallo-beta-lactamase (MBL) producers' demonstrated 100% resistance to Gentamicin, Cefotaxime, and Imipenem. Additionally, the isolates displayed a 5.55% resistance rate to Polymyxin B, as detailed in Table 1.

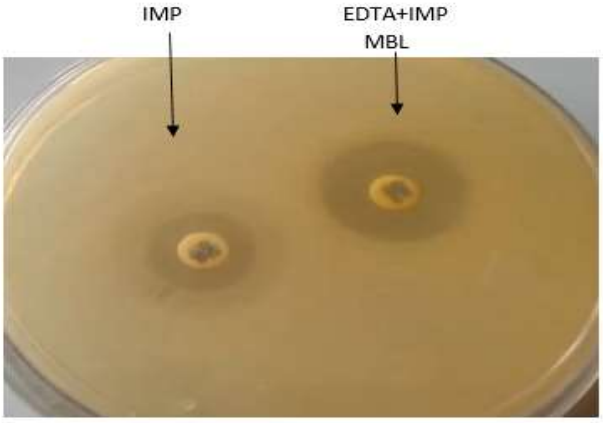

Figure 1: Imipenum –EDTA combine –disk synergy test IMP-EDTA*Imipenem -EDTA

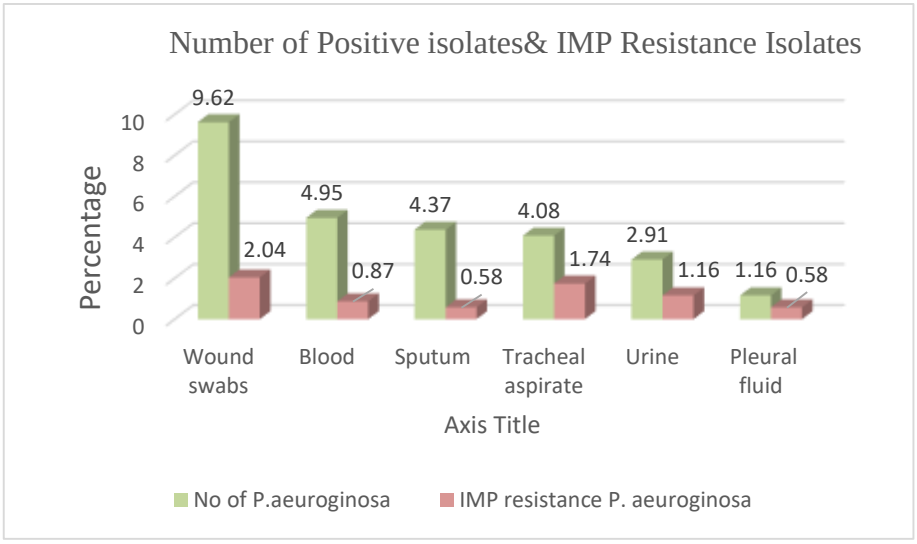

Figure 2: Distribution of various specimens and IMR resistance of *P. aeruginosa*

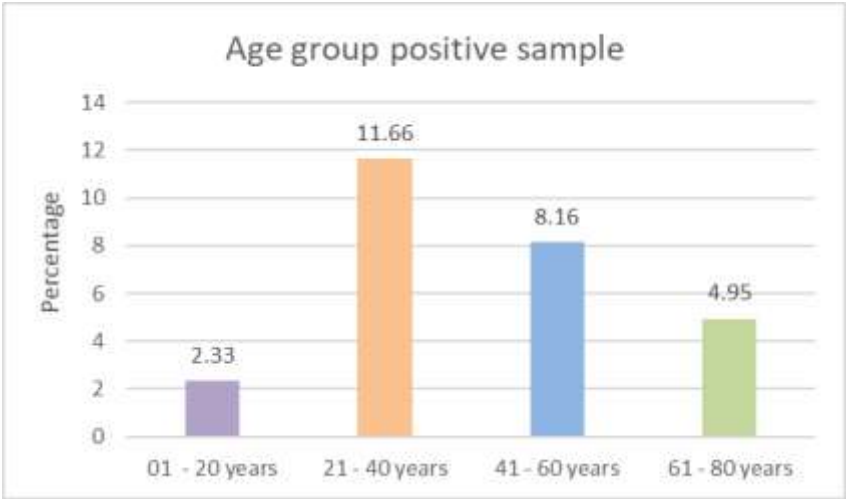

Figure 3: Age wise distribution of Positive samples

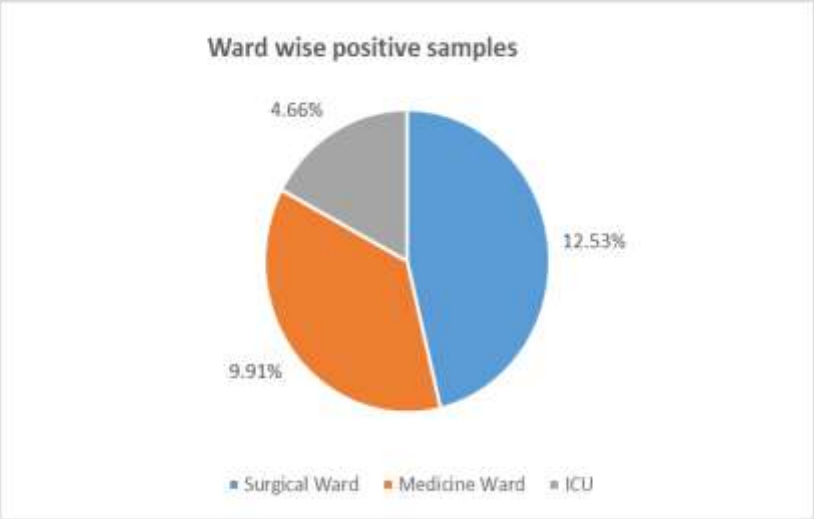

Figure 4: Wards wise distribution of Positive samples

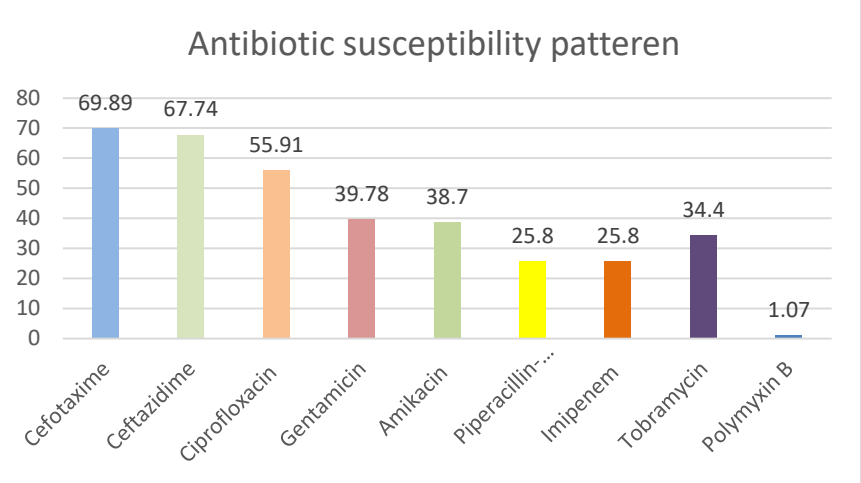

Figure 5: Resistance profile of *P. aeruginosa* against various Antibiotics

Antibiotics	MBL Positive (n=18)		MBL Negative (n= 6)	
	isolates	%	isolates	%
Cefotaxime	18	100	6	100
Ceftazidime	18	100	6	100
Gentamicin	18	100	5	83.33
Amikacin	15	83.33	4	66.66
Tobramycin	15	83.33	3	50
Ciprofloxacin	13	72.22	3	50
Imipenem	18	100	6	100
Piperacillin-Tazobactam	12	66.66	4	66.66
Polymixin B (PB)	1	5.55	0	0

Table 1: Antibiotic resistance profiles of isolates MBL positive and negative producer.

DISCUSSIONS

In our study, the incidence of *P. aeruginosa* isolates in clinical samples from the 21-40 age group was notably high, observed at 43.01%. A similar observation was studied by[13,14]. Moreover, contrast with our study[15] indicated a higher incidence of *P. aeruginosa* in patients aged 41 to 60 years. Whereas in present study incidence of male with *P. aeruginosa* was (58.06 %) while females had (44.93 %). This is comparable with study of [16].

In present study the highest percentage (46.23%) of *P. aeruginosa* incidence was observed in the surgical ward, followed by medicine ward (36%) and ICU (17.20%). These results are in line with studies of[17]. While the study of [18] showed a slight contradiction in the incidence of cases in surgical wards, medical wards, and the ICU, the findings regarding *P. aeruginosa* were significant

In present study, the highest incidence was observed in wound swabs (35.46%), followed gradually by blood (18.27%), sputum (16.12%), tracheal aspirates (15.05%), urine (10.75%), and pleural fluids (4.30%) for *P. aeruginosa* infection. A similar observation made by[13] Showing highest incidence in wound swab 40 %, with sputum accounting for 18.3%, urine for 10%, and body fluids for 3.3%, while study of [19] showed maximum isolates in urine samples 36 % followed by wound swabs 28 %, blood 14 %, sputum10 % and tracheal aspirates 8 %.

The antibiotic susceptibility pattern of 83 isolates of *P. aeruginosa* revealed the highest resistance to Cefotaxime at 69.89%, subsequent to Ceftazidime at 67.74%. Ciprofloxacin 55.91% Gentamicin 39.78 %, Amikacin 38.70 %, Piperacillin-Tazobactum 25.80 % and Imipenem 25.80 % were resistance, similarly to the observation done by [13] A different resistance pattern against Cefotaxime 90 % and Ceftazidime 85 % was showed [20].

According to this study, *P. aeruginosa* are become increasingly resistant to new antibiotics and less susceptible to widely used antibiotic. The spread of resistant organisms as a result of inappropriate antibiotic usage, ignorance, patient noncompliance, and poor hygiene is rendering antimicrobial drugs ineffective.

In our study we found 25.80 %resistance to imipenem. The dissimilarity study by [21] showed that 49.5% of *P. aeruginosa* strains were resistant to imipenem. Most MBL positive isolates in our study were recovered from patients between 20 and 40 years of age with wounds as their main source. This is similar with previous study [21].

In the present study, we observed a 19.35% incidence of MBL-producing *P. aeruginosa* among the clinical isolates, which aligns with similar findings reported by[22] where 20% were found to be MBL producers. Additionally, a recent study conducted by[13] corroborated these findings by also investigating the prevalence of MBL-producing strains.

Conclusion:

Metallo-Beta-Lactamase (MBL) production plays a key role in Carbapenem resistance in *P. aeruginosa*. Early diagnosis using sensitive techniques like the Combined Disc Synergy Test with Imipenem (CDST-IPM) is crucial to prevent resistant strain spread and reduce mortality. The integration of molecular detection methods with routine phenotypic assays can further enhance diagnostic accuracy. Continuous surveillance and strict infection control practices are essential to limit the emergence and dissemination of multidrug-resistant strains.

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