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Research Article

PREVALENCE OF MBL PRODUCING PSEUDOMONAS AERUGINOSA IN TERTIARY CARE HOSPITAL

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Abstract
Pseudomonas aeruginosa, a notorious nosocomial pathogen, exhibits intrinsic resistance to various antibiotics. The emergence of Metallo-β-lactamase (MBL) production in *P. aeruginosa* poses a serious threat to the efficacy of carbapenems, crucial antibiotics in treating severe infections. Tertiary care hospitals, due to their complexity and patient diversity, are potential reservoirs for multidrug-resistant organisms, making the investigation of MBL prevalence imperative for informed therapeutic interventions. This study aims to determine the prevalence of MBL-producing *P. aeruginosa* in a tertiary care hospital, elucidating the epidemiology, antibiotic sensitivity patterns, and potential implications for patient outcomes. Clinical isolates of *P. aeruginosa* will be collected seventy samples from diverse hospital wards over two months. The presence of MBL will be assessed using established phenotypic methods, and antibiotic sensitivity profiles will be determined. Statistical analyses will be employed to evaluate the prevalence rates and associations. The percentage and Mean ±SD were used to express the data. The study anticipates revealing the frequency of MBL production in *P. aeruginosa* isolates, providing insights into the distribution across hospital wards. Antibiotic sensitivity patterns will be elucidated, emphasizing the potential impact on therapeutic strategies. Understanding the prevalence of MBL-producing *P. aeruginosa* in a tertiary care hospital setting is crucial for optimizing antimicrobial stewardship and infection control measures. The findings of this study are anticipated to contribute valuable insights into the current landscape of antibiotic resistance, guiding evidence-based interventions to enhance patient care and minimize the spread of multidrug-resistant pathogens in healthcare settings.

Keywords:- *Pseudomonas aeruginosa*, Metallo-β-lactamase (MBL), Prevalence, Antibiotic resistance, Antibiotic sensitivity patterns, Multidrug-resistant organisms.

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INTRODUCTION

Infections caused by Pseudomonas aeruginosa have emerged as a significant public health concern, especially in healthcare settings where immunocompromised patients are more susceptible to opportunistic pathogens. P. aeruginosa is known for its intrinsic resistance to multiple antimicrobial agents, and the emergence of Metallo-β-lactamase (MBL) production in this bacterium further complicates treatment options [1-3]. MBLs are enzymes that confer resistance to carbapenems, a class of antibiotics often considered the last resort for treating severe bacterial infections. The prevalence of MBL-producing P. aeruginosa strains has become a focal point for research, as it poses a serious threat to the efficacy of available antibiotics [4-5].

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Understanding the prevalence of MBL production in *P. aeruginosa* is crucial for guiding empirical antibiotic therapy and implementing effective infection control measures in healthcare settings. Tertiary care hospitals, due to their complexity and the nature of patients they serve, are particularly susceptible to the spread of multidrug-resistant organisms. Therefore, investigating the prevalence of MBL-producing P. aeruginosa in a tertiary care hospital setting is essential for informing targeted interventions and ensuring optimal patient outcomes [6-7].

Pseudomonas aeruginosa is an opportunistic pathogen associated with nosocomial infections, and its ability to develop resistance to multiple antibiotics poses a serious challenge for clinicians. Carbapenems, such as imipenem and meropenem, are often considered the last line of defense against P. aeruginosa infections. However, the emergence of Metallo-β-lactamase (MBL) production in P. aeruginosa strains compromises the efficacy of these critical antibiotics [8-9].

Tertiary care hospitals, being hubs for complex medical interventions and catering to a diverse patient population, are at an elevated risk of harboring antibiotic-resistant strains. The prevalence of MBL-producing P. aeruginosa in such settings can serve as a key indicator of the extent of antimicrobial resistance and guide the development of targeted therapeutic strategies [10-13].

This study aims to investigate the prevalence of MBL-producing P. aeruginosa in a tertiary care hospital, shedding light on the epidemiology of this resistance mechanism. The research will involve the analysis of clinical isolates to determine the frequency of MBL production, antibiotic sensitivity patterns, and the potential impact on patient outcomes. By identifying the prevalence of MBL-producing P. aeruginosa in a tertiary care setting, this study intends to contribute valuable insights for optimizing antimicrobial stewardship programs, enhancing infection control measures, and ultimately improving the quality of patient care.

MATERIAL AND METHODS

This research was carried out in Bhaarath Medical College and Hospital,Chennai. It's an analytical, prospective research. The trial will last for two months.
Number of samples: Seventy P.aeruginosa isolates at least.

Qualifications for inclusion:

All P.aeruginosa isolates from patients hospitalized to tertiary care hospitals, spanning all age groups and genders, were included in this investigation.

Criteria for exclusion:

Individuals visiting outpatient clinics (OPD) isolated from the same patients over and again. Sputum, bronchoalveolar lavage (BAL), endotracheal aspirates, urine, blood, wound swabs, and indwelling catheters were among the samples that were collected in fully aseptic circumstances.

Oxidase reaction, colony morphology on Blood agar and Mac Conkey's agar, pigment synthesis, citrate utilisation, sugar fermentation, TSI reaction, and polymyxin B sensitivity tests are used to identify Pseudomonas aeruginosa isolates.

Muller-Hinton agar plates were subjected to antimicrobial sensitivity testing using the Kirby-Bauer disc diffusion technique. The disc diffusion method was used to test the following antibiotics (Hi-Media, India): imipenem (10 µg), gentamicin (10 µg), netilmicin (30 µg), polymyxin-B (300 units), colistin (10 µg), and piperacillin/tazobactam (100 µg/ 10 µg). For quality control, P. aeruginosa ATCC 27853 was employed [14-15].

Comparing several phenotypic techniques for P. aeruginosa MBL production detection has also been done. Calculating percentages and basic ratios was the method used for statistical analysis. The percentage and Mean ±SD were used to express the data.

Imipenem (IMP)-EDTA combined disc test

The combined disc method was performed as described byYong *et al* . A lawn culture of the test isolate will beprepared. After allowing it to dry for 5 minutes, twoimipenemiscs one with 0.5 M EDTA and the other plainwere placed on the surface of the agar plate approximately30mm apart. The plates were incubated overnight at 37 oC.An increase in the zone diameter of ≥ 7 mm aroundimipenem+EDTA disc in comparison to imipenem discalone indicated production of MBL.

Imipenem-EDTA double disc synergy test (DDST)

The IMP-EDTA double disk synergy test was performed as described by Lee *et al*.(24) Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI. An imipenem (10 µg) disc was placed 20 mm centre to centre from a blank disc containing 10 µL of 0.5 M EDTA (750 µg). Enhancement of the zone of inhibition in the area between imipenem and the EDTA disc in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result [16-17].

RESULTS

The study was conducted in a tertiary care facility in January and February of 2022. The study was conducted to determine the frequency of MBL generation in P.aeruginosa isolates, their pattern of antibiotic sensitivity, and the corresponding mortality among infected patients in our hospital. Comparing several phenotypic techniques for P. aeruginosa MBL production

detection has also been done. The following tables and figures show the findings of the analysis and presentation.

Among all inpatients number of cases of *P. aeruginosa* infection was highest in surgical ward, followed by orthopedic wards.

P. aeruginosa strains were isolated most commonly from pus & exudates, followed by other samples.

Among the 70 patients harboring P. aeruginosa infection, 58(82.8%) were male and only 12 (17%) were female

All 49imipenem resistant P. aeruginosa strains were recovered from male patients.

Out of total 70 isolates of *P. aeruginosa* screened for MBL production by imipenem disk diffusion test, 39 isolates (56%) were imipenem resistant and rest 31 (44%) were sensitive to imipenem.

All 21*P. aeruginosa* strains showing resistance to imipenem10 µg disk in screening test, were further tested by disksynergy test & combined disk test.

Table.No. 1: Distribution of P. aeruginosa in hospital wards.

Sources of *P. aeruginosa* isolates	Number of isolates (n=70)	Percentage
General surgery	29	41.4
Orthopedic	12	17.1
ENT	7	10
ICU	5	7.1
General medicine	5	7.1
Tb and chest disease	4	5.7
OBG &GYN	3	4.2
Pediatric surgery	3	4.2
Urology	2	2.8

Table.No. 2: Distribution of isolates in clinical samples

Clinical samples	Number of isolates (n=70)	Percentage
Pus	28	40
Sputum	12	17
Pus aspiration	9	12.8
Et secretion	7	10
Urine	6	8.5
Bronchial aspiration	5	7.1
Vaginal swab	3	4.2

Table 3: Gender distribution of patients infected with Ps. Aeruginosa.

Gender	Number of isolates (n=70)	Percentage
Male	58	82.8
Female	12	17

Table 4: Imipenim resistance in P.aeruginosa strains.

Antibiotics	Number of isolates (n=70)	Percentage
Imipenem sensitive	31	44
Imipenem resistance	39	56

Table 5: Comparison of disk synergy test & combined disk test for detection of MBL producing strains.

Test method	Number of isolates (n=70)	Percentage
Imipenem +EDTA combind disk test	21	30
Disk synergy test	10	14.2

DISCUSSION

Our study aimed to assess the prevalence of Metallo-β-lactamase (MBL) generation in Pseudomonas aeruginosa isolates, determine their antibiotic sensitivity patterns, and evaluate associated mortality among infected patients. Additionally, we compared various

phenotypic techniques for detecting MBL production in P. aeruginosa. Our study provide a comprehensive overview of the distribution of P. aeruginosa in different hospital wards, clinical samples, gender distribution of infected patients, and the resistance profile to imipenem.

Our study illustrates the distribution of P. aeruginosa isolates across various hospital wards. The highest number of isolates was found in the General Surgery ward (41.4%), followed by Orthopedic (17.1%) and ENT (10%) wards [18-19]. This highlights a significant prevalence of P. aeruginosa infections in surgical departments.In India, prevalence rate of P.aeruginosa infection varies from 10.5% to 30%. It ranged from 3 to 16%, in a multicentric study conducted by Ling J M et al.

Present study provides insights into the distribution of P. aeruginosa isolates in different clinical samples. Pus and exudates were the most common sources of isolates (40%), followed by sputum (17%) and pus aspiration (12.8%). This emphasizes the predilection of P. aeruginosa for wound and respiratory tract infections [20-21].*P.aeruginosa* were predominantly isolated from pus (47.11%), followed by sputum sample (36.53%). The same has been reported with Okon et al., (39.2%) [6], &VijayaChaudhari et al., (35.3%). A recent study from Tehran, Iran has reported a very high prevalence of 94.2% Metallo-beta-lactamases producing P. aeruginosa in burn patients.

Our study reveals a notable gender disparity among patients harboring P. aeruginosa infections, with 82.8% being male and only 17% female. This observation suggests a potential gender-associated susceptibility to P. aeruginosa infections, warranting further investigation [22-23].

The resistance profile of P. aeruginosa strains to imipenem. Among the 70 isolates, 56% demonstrated resistance to imipenem, while 44% remained sensitive. Intriguingly, all imipenem-resistant strains were recovered from male patients, indicating a potential gender-specific resistance pattern [24-25].

Our study compares two phenotypic techniques for detecting MBL-producing strains: Imipenem + EDTA combined disk test and Disk Synergy test. Among the tested isolates, 30% were positive for MBL production using the combined disk test, while 14.2% were positive with the Disk Synergy test [26-27]. This highlights the importance of choosing appropriate methods for accurate detection of MBL production.Hemlatha et al. (2005) from India reported that 16% of P. aeruginosa isolates were resistant to imipenem and 14% were positive for MBL production by combined disk test (Hemlatha et al., 2005). Behera et al. (2008) found 14.47% of P. aeruginosa resistant to imipenem and 10.53% positive for MBL production by combined-disk test. Behera et al. (2008) reported 7.2% imipenem resistance and 4.4% MBL positivity by combined-disk test among P. aeruginosa isolates (Berges et al., 2007).

The observed high prevalence of P. aeruginosa infections in surgical wards, particularly among male patients, underscores the importance of stringent infection control measures in these settings. The resistance of P. aeruginosa strains to imipenem raises concerns about the limited efficacy of this crucial antibiotic. The gender disparity in infection rates prompts further research to explore underlying factors contributing to susceptibility [9,13,15].

Furthermore, the variation in MBL detection rates between the tested methods emphasizes the need for standardized and reliable techniques in clinical settings. The findings from this study can guide clinicians in selecting appropriate diagnostic approaches and contribute to the development of targeted therapeutic strategies, ultimately improving patient outcomes and reducing the impact of P. aeruginosa infections in hospital settings.

It is essential to acknowledge certain limitations, such as the single-center nature of the study and the need for a larger, multicenter investigation to validate the findings. Future research should explore the molecular mechanisms underlying imipenem resistance and evaluate the clinical outcomes associated with MBL-producing P. aeruginosa infections. These insights are crucial for refining treatment strategies and minimizing the morbidity and mortality associated with these infections.

CONCLUSION

Our study provides valuable insights into the epidemiology, resistance patterns, and detection methods of P. aeruginosa infections. These findings contribute to the ongoing efforts to optimize clinical management strategies and mitigate the impact of resistant bacterial strains in healthcare settings.

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