Prevalence and Phenotypic Detection of Metallo-β-Lactamase-Producing *Pseudomonas aeruginosa* in Clinical Settings: A Prospective Study from Medical City \ Baghdad Teaching Hospital

Abstract

Background: The global surge in multidrug-resistant organisms has rendered Metallo-β-Lactamase (MBL)-producing Pseudomonas aeruginosa a serious threat to healthcare systems. This study aimed to assess the prevalence of MBL-producing P. aeruginosa strains and evaluate the efficacy of phenotypic detection methods.

Methods: A cross-sectional study was conducted between July 2023 and July 2024 in Teaching Hospital Baghdad/ Medical City. A total of 229 *P. aeruginosa* isolates were collected from various clinical specimens. Identification and antibiotic susceptibility testing were performed using conventional and automated systems. Carbapenem-resistant isolates were further tested for MBL production using the Combined Disk Test (CDT) and E-test.

Results: Out of 229 isolates, 44 (19.2%) were resistant to carbapenems. Among these, 30 (67.5%) were confirmed as MBL producers using both CDT and E-test. MBL-producing strains were more frequently isolated from patients over 60 years of age, predominantly from pus (40.2%) and urine (28.3%) samples.

Conclusion: The significant presence of MBL-producing P. aeruginosa emphasizes the need for routine phenotypic screening and robust antimicrobial stewardship programs. CDT and E-test remain accessible and reliable tools for early detection, especially in resource-constrained settings.

Keywords: Pseudomonas aeruginosa, Metallo-β-Lactamase, carbapenem resistance.

1. Introduction

Antimicrobial resistance (AMR) poses a significant global threat to public health, particularly in hospital settings. Among Gram-negative pathogens, Pseudomonas aeruginosa stands out due to its intrinsic resistance mechanisms and its ability to acquire additional resistance genes via horizontal gene transfer [1]. A particularly worrisome resistance mechanism is the production of metallo-β-lactamases (MBLs), enzymes that confer resistance to almost all β-lactam antibiotics, including carbapenems—the last-resort antibiotics used for severe infections [2, 3].

MBLs belong to Ambler class B β-lactamases and require divalent metal ions, typically zinc, to function. Genes encoding these enzymes are often

located on mobile genetic elements such as plasmids and integrons, facilitating their rapid dissemination among bacterial populations [4]. Several MBL types have been identified globally, including IMP, VIM, SPM, and GIM, with significant clinical impact reported tertiary care center in Teaching Hospital Baghdad/Medical city, Iraq [5].

Despite the urgent need for accurate detection, there is currently no universally accepted gold standard for MBL identification. While molecular techniques such as PCR provide definitive genotypic confirmation, their cost and complexity often limit their use in routine diagnostics, especially in resource-limited regions [6]. Consequently, phenotypic methods—such as (CDT) and E-test—remain widely used due to their affordability and ease of use [7].

This study aimed to determine the prevalence of MBL-producing P. aeruginosa in a tertiary care center in Teaching Hospital Baghdad/Medical city, and to evaluate the utility of CDT and E-test as phenotypic detection tools.

2. Materials and Methods

2.1 Study Design and Setting

This cross-sectional study was conducted over a 12-month period, from July 2023 to July 2024, at a tertiary care center in Teaching Hospital Baghdad/Medical city, Iraq. Ethical approval was obtained from the institutional review board, and all procedures adhered to standard clinical laboratory protocols.

2.2 Sample Collection

A total of 229 clinical specimens were collected aseptically from hospitalized patients. The specimens included pus, urine, blood, sputum, endotracheal secretions, cerebrospinal fluid (CSF), and catheter tips. Samples were promptly transported to the microbiology laboratory for culture and identification.

2.3 Isolation and identification

Isolation and identification of P. aeruginosa were carried out using both conventional and automated systems. Traditional methods included assessment of colony morphology, pigment production, oxidase test, motility, and growth at 42°C. The VITEK 2 Compact System (bioMérieux, France) was used for automated identification and confirmation.

2.4 Antimicrobial Susceptibility Testing

Antibiotic susceptibility was assessed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, following Clinical and Laboratory Standards Institute (CLSI) guidelines [8]. The antibiotics tested included imipenem, meropenem, ceftazidime, cefepime, piperacillin-tazobactam, amikacin, gentamicin, ciprofloxacin, colistin, and polymyxin B.

2.5 Phenotypic Detection of MBL

All isolates that showed resistance to carbapenems (imipenem or meropenem) were subjected to phenotypic confirmatory tests for MBL detection:

- Combined Disk Test (CDT): Imipenem and ceftazidime disks with and without 0.5 M EDTA were placed on Mueller-Hinton agar. An increase of ≥7 mm in zone diameter in the presence of EDTA was considered indicative of MBL production [1].
- E-test: Ezy MIC™ strips containing meropenem and meropenem+EDTA were used. A ≥8-fold reduction in MIC or the appearance of a phantom zone indicated MBL activity [9].

2.6 Quality Control

P. aeruginosa ATCC 27853 was used as the negative control for both susceptibility and MBL detection assays.

3. Results

A total of 229 Pseudomonas aeruginosa isolates were obtained from diverse clinical specimens, including pus (n = 92, 40.2%), urine (n = 65, 28.4%), blood (n = 26, 11.4%), endotracheal secretions (n = 19, 8.3%), sputum (n = 11, 4.8%), throat swabs (n = 3, 1.3%), and cerebrospinal fluid (n = 1, 0.4%) (Table 1, Figure 1).

Out of the 229 isolates, 44 (19.2%) were resistant to carbapenems (imipenem and/or meropenem). These carbapenem-resistant strains were subjected to phenotypic testing for MBL production using the (CDT) and E-test.

Among the 44 carbapenem-resistant isolates, MBL production was confirmed in 30 isolates. Of these, 24 (80%) tested positive by both imipenem+EDTA and ceftazidime+EDTA disks in CDT, while the remaining 6 (20%) were positive with imipenem+EDTA only. All 30 isolates showed concordant positive results in the E-test, confirming the presence of MBL activity through a ≥8-fold MIC reduction or phantom zone appearance in the presence of EDTA.

These findings indicate a substantial burden of MBL-producing P. aeruginosa in the clinical setting, particularly from pus and urine samples.

4. Discussion

The increasing prevalence of Metallo-β-Lactamase (MBL)-producing Pseudomonas aeruginosa represents a critical challenge in hospital-acquired infections due to limited treatment options and increased morbidity. In this study, 44 out of 229 (19.2%) isolates were resistant to carbapenems, and 30 (67.5%) of these were phenotypically confirmed as MBL producers. These findings align with regional data, which report MBL prevalence rates ranging from 30% to 70% among carbapenem-resistant P. aeruginosa isolates [8,9].

Our data also revealed that the majority of MBL-producing strains originated from pus (40.2%) and urine (28.3%) samples, indicating a high prevalence of resistant organisms in wound and urinary tract infections. These reservoirs are particularly concerning in hospitalized and immunocompromised patients, especially the elderly, who are at elevated risk of infection and adverse outcomes [10].

Phenotypic methods such as the Combined Disk Test (CDT) and E-test demonstrated excellent concordance in detecting MBL-producing isolates, consistent with previous research validating their utility in clinical laboratories [1,4,7]. CDT remains a low-cost, accessible method suitable for routine screening, while the E-test offers enhanced precision in determining minimum inhibitory concentrations (MICs), which supports informed clinical decisions.

Although molecular techniques such as PCR and gene sequencing remain the gold standard for identifying specific MBL genotypes, they are not always feasible in resource-limited environments [6]. Therefore, phenotypic assays remain essential tools in such contexts to facilitate timely infection control and appropriate antimicrobial therapy.

Limitations of this study include the lack of molecular confirmation for MBL genes and the absence of comprehensive clinical data, such as patient comorbidities and treatment outcomes. Future research should integrate genotypic analysis and explore the relationship between resistance profiles and clinical variables to provide a more robust epidemiological understanding.

5. Conclusion

This study highlights a notable prevalence of MBL-producing Pseudomonas aeruginosa among clinical isolates in a tertiary care center in Teaching

Hospital Baghdad/Medical city, Iraq.. Out of 229 isolates, 19.2% were resistant to carbapenems, and 67.5% of those were confirmed as MBL producers through phenotypic methods.

Routine screening for MBL production using affordable and accessible tools such as the Combined Disk Test (CDT) and E-test remains vital, particularly in resource-constrained settings. These methods not only support appropriate antimicrobial therapy but also play a crucial role in infection control and limiting the spread of multidrug-resistant pathogens.

Future studies incorporating molecular techniques and clinical outcome data are essential to further elucidate the epidemiology and clinical significance of MBL-producing organisms in this region.

6. References

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