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bla_{VIM} mediated carbapenem resistance in *Pseudomonas aeruginosa*

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Abstract:
Carbapenem-Resistant *Pseudomonas aeruginosa* (CRPA) is becoming a growing problem in tertiary healthcare facilities with the acquisition of metallo-beta-lactamase (MBL) genes, specifically the Verona Integron-encoded Metallo-beta-lactamase (VIM) gene, making standard 37 -lactam-based treatment of this pathogen ineffective. A total of 240 clinical isolates of *P. aeruginosa* of a high-volume intensive care unit were analyzed in this cross-sectional study to assess the antimicrobial susceptibility profiles and molecularly characterize the prevalence of bla_{VIM} variants by PCR amplification. Phenotypic study showed resistance to carbapenem as 42.5 percent and molecular genotyping showed that 68.6 percent of the resistant groups carried bla_{VIM} that was statistically significant in co-resistance with aminoglycoside and fluoroquinolones relative to VIM-negative strains. Thus, supremacy of the bla_{VIM}-mediated resistance indicates the significant endangered state of health, so the urgent necessity is to implement molecular screening guidelines and limit empirical use of carbapenems.

Keywords: *Pseudomonas aeruginosa*, bla_{VIM}, carbapenem resistance, metallo-β-lactamase (MBL), nosocomial infections

Background:
Pseudomonas aeruginosa is an omnipresent opportunistic Gram-negative pathogen, causing serious healthcare-associated infections, such as ventilator-associated pneumonia (VAP), bloodstream infections and complicated urinary tract infections. The presence of intrinsic resistance mechanisms and exceptional ability to develop adaptive resistance has led the World Health Organization (WHO) to categorise carbapenem-resistant *P. aeruginosa* as a high priority pathogen that urgently needs new therapeutic agents to be developed **[1]**. As the mainstay of treatment against severe cases of pseudomonal infections, carbapenems, mostly imipenem and meropenem were used in the past, but recently all over the world, there is an increasing rate of resistance undermining the clinical use of the latter **[2]**. *P. aeruginosa* resistance to carbapenems is complex, but it is usually a combination of chromosomal mutations that cause OprD porin loss, the up-regulation of MexAB-OprM efflux pumps and the most worrying aspect of all, there is production of carbapenem-hydrolyzing enzymes (carbapenases) **[3]**. The most problematic of these enzymes are Metallo- 2 -lactamases (MBLs) of Ambler Class B. The MBLs use zinc ions in the active site compared to serine carbapenemases (like KPC) to break down the z -lactam ring. This structural difference makes them resistant to clinically available β-lactamase inhibitors, such as clavulanic acid, tazobactam and avibactam **[4]**. One of the most common MBL families in the world is known as the Verona Integron-encoded Metallo- 2 -lactamase (VIM). Bla_{VIM} gene has experienced massive genetic evolution and spread across the world, since it was first discovered in Verona, Italy. The gene is normally present inside class 1 integrons which are mobile genetic elements that have the ability to capture and express gene cassettes. This genetic background has been clinically catastrophic since these integrons are often also harboring other resistance determinants against aminoglycosides and sulfonamides, producing Multi-Drug Resistant (MDR), Extensively Drug-Resistant (XDR) phenotypes **[5]**. Recent epidemiological surveillance has suggested a change in the geographical localization of VIM whereby rising cases are being reported in Southern Europe, Middle East and some regions of Asia. Although more recent agents, including ceftolozane-tazobactam and ceftazidime-avibactam, have been shown to work more favorably in some resistant phenotypes, there are no solutions to MBL producers, including VIM-positive strains and clinicians are only left with cefiderocol or colistin **[6]**. Therefore, it is of interest to describe the prevalence of bla_{VIM}-mediated resistance in clinical isolates of *Pseudomonas aeruginosa* and to compare the phenotypic antimicrobial susceptibility patterns between VIM-producing and non-VIM-producing strains in the studied population.

Materials and Methods:
Study design and setting:
This cross-sectional, laboratory-based observational study was conducted over a period of 14 months at the Department of Microbiology in a 1,200-bed academic tertiary care center. The hospital includes distinct medical, surgical and pediatric Intensive Care Units (ICUs), oncology wards and a burn unit.

Sample collection and bacterial identification:
A total of 240 non-duplicate clinical isolates of *Pseudomonas aeruginosa* were collected consecutively from various clinical specimens (sputum, endotracheal aspirates, blood, urine, wound swabs and catheter tips). Duplicate isolates from the same patient within a 30-day window were excluded to prevent data skewing. Initial isolation was performed on Blood Agar and MacConkey Agar. Identification was confirmed using standard biochemical tests (oxidase positive, growth at 42°C, pigment production) and validated using the VITEK® 2 Compact System (bioMérieux, Marcy-l'Étoile, France) using GN ID cards.

Antimicrobial Susceptibility Testing (AST):
Antimicrobial susceptibility was determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (MHA) in strict accordance with the Clinical and Laboratory Standards Institute (CLSI) M100 performance standards (2024 edition). The antibiotic panel included: Piperacillin-Tazobactam (100/10 µg), Ceftazidime (30 µg), Cefepime (30 µg), Imipenem (10 µg), Meropenem (10 µg), Gentamicin (10 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg) and Aztreonam (30 µg). For Colistin and Polymyxin B, Minimum Inhibitory Concentrations (MIC) was determined using the broth microdilution (BMD) method, as disk diffusion is inadequate for polymyxins. Results were interpreted as Susceptible, Intermediate, or Resistant based on CLSI breakpoints. *P. aeruginosa* ATCC 27853 was used as a quality control strain.

Phenotypic detection of MBLs:
Isolates showing resistance or intermediate susceptibility to imipenem or meropenem were screened for MBL production using the Imipenem-EDTA Combined Disk Test (CDT). Two imipenem disks (10 µg) were placed on MHA inoculated with the test organism. One disk was potentiated with 10 µL of 0.5 M EDTA solution. An increase in zone diameter of 7 mm around the Imipenem-EDTA disk compared to the Imipenem disk alone was considered positive for MBL production.

Molecular characterization of bla_{VIM}:
Genomic DNA was extracted from fresh overnight cultures using the boiling lysis method. The supernatant was used as the template for Polymerase Chain Reaction (PCR). Specific primers targeting the conserved regions of the bla_{VIM} lineage were utilized.

[1] Forward Primer: 5′-GTTTGGTCGCATATCGCAAC-3′

[2] Reverse Primer: 5′-AATGCGCAGCACCAGGATAG-3′

PCR amplification was performed in a thermal cycler with the following conditions: initial denaturation at 95°C for 5 min; 30 cycles of denaturation at 95°C for 30s, annealing at 56°C for 40s and extension at 72°C for 50s; followed by a final extension at 72°C for 5 min. Amplified products (approx. 382 bp) were visualized on 1.5% agarose gel electrophoresis stained with ethidium bromide.

Statistical analysis:
Data were analyzed using IBM SPSS Statistics for Windows, Version 26.0. Continuous variables were presented as mean ± standard deviation (SD), while categorical variables were expressed as frequencies and percentages. The Chi-square (χ^2) test or Fisher's exact test was used to compare resistance rates between VIM-positive and VIM-negative groups. A p-value of <0.05 was considered statistically significant.

Results:
Of the 240 *P. aeruginosa* isolates collected, the majority were obtained from male patients (62.9%, n=151). The mean age of the patient population was 56.4 ± 14.2 years. The highest number of isolates originated from the Intensive Care Units (ICU), accounting for 55.0% of the total sample, followed by surgical wards (25.0%). Respiratory tract specimens (Endotracheal aspirates and sputum) constituted the most frequent source of isolation (45.8%), reflecting the high burden of ventilator-associated pneumonia **(Table 1).** The overall resistance profile demonstrated high levels of resistance to β -lactams. Carbapenem resistance was observed in 102 isolates (42.5%) for Meropenem and 108 isolates (45.0%) for Imipenem. Among non-β -lactams, Amikacin showed the best activity with 72.5% susceptibility, whereas Ciprofloxacin resistance was high at 40.8%. Colistin retained excellent activity, with 98.3% of isolates being susceptible **(Table 2).** All 102 isolates identified as Meropenem-resistant were subjected to phenotypic MBL screening and PCR. The phenotypic CDT was positive in 78 isolates (76.5% of resistant strains). PCR analysis confirmed the presence of the bla_{VIM} gene in 70 of the carbapenem-resistant isolates (68.6%). When comparing the antibiotic resistance profiles of bla_{VIM} positive isolates against Carbapenem-Resistant bla_{VIM} negative isolates (likely resistant via porin loss or efflux pumps), the bla_{VIM} positive group exhibited significantly higher rates of co-resistance to other antibiotic classes, particularly aminoglycosides and fluoroquinolones **(Table 3).**

Table 1: Demographic characteristics and specimen distribution (N=240)

Parameter	Category	Frequency (n)	Percentage (%)
Gender	Male	151	62.9%
	Female	89	37.1%
Patient Location	ICU (Medical/Surgical)	132	55.0%
	Surgical Wards	60	25.0%
	Medical Wards	36	15.0%
	Outpatient/Other	12	5.0%
Specimen Type	Respiratory Tract	110	45.8%
	Pus/Wound Swab	55	22.9%
	Urine	45	18.8%
	Blood	30	12.5%

Table 2: Overall antimicrobial resistance profile of *P. aeruginosa* (N=240)

Antibiotic Class	Antibiotic Agent	Resistant n (%)	Intermediate n (%)	Sensitive n (%)
Carbapenems	Meropenem	102 (42.5%)	10 (4.2%)	128 (53.3%)
	Imipenem	108 (45.0%)	8 (3.3%)	124 (51.7%)
Cephalosporins	Ceftazidime	98 (40.8%)	12 (5.0%)	130 (54.2%)
	Cefepime	95 (39.6%)	15 (6.2%)	130 (54.2%)
Penicillins	Pip/Tazobactam	90 (37.5%)	18 (7.5%)	132 (55.0%)
Aminoglycosides	Amikacin	50 (20.8%)	16 (6.7%)	174 (72.5%)
	Gentamicin	85 (35.4%)	5 (2.1%)	150 (62.5%)
Fluoroquinolones	Ciprofloxacin	98 (40.8%)	10 (4.2%)	132 (55.0%)
Polymyxins	Colistin	4 (1.7%)	-	236 (98.3%)

Table 3: Comparison of resistance rates between bla_{VIM} positive and bla_{VIM} negative carbapenem-resistant isolates (N=102)

Antibiotic	bla_{VIM} Positive (n=70)	bla_{VIM} Negative (n=32)	P-value
Ceftazidime	70 (100%)	28 (87.5%)	0.003
Cefepime	68 (97.1%)	27 (84.4%)	0.018
Pip/Tazobactam	65 (92.9%)	25 (78.1%)	0.032
Gentamicin	60 (85.7%)	12 (37.5%)	<0.001
Amikacin	45 (64.3%)	5 (15.6%)	<0.001
Ciprofloxacin	62 (88.6%)	18 (56.3%)	<0.001
Aztreonam	48 (68.6%)	15 (46.9%)	0.035

Discussion:
The spread of carbapenem-resistant *P. aeruginosa* (CRPA) across the world in a very short period of time is a medical crisis. Our experiment proposes an alarming amount of the bla_{VIM} metallo-2 -lactamase gene in our clinical environment, with 68.6% of carboxymethylpenicillin-resistant isolates bearing this determinant. This can be related to the latest epidemiological reports that VIM is the most common MBL in certain parts of Europe or the Mediterranean basin **[7]**. The total rate of carbapenem resistance (42.5) observed in our analysis is greater than the national prevalence rates (in North America) but in line with the high-prevalence areas that are explicated in the 2023 SENTRY surveillance publications **[8]**. The clinical background of bla_{VIM} detection is that it predetermines therapeutic failure of numerous standard and innovative therapies. Though the precise mechanisms of the VIM-negative resistant isolates were outside the focuses of the present study, it is postulated in the literature that the OprD porin or overexpression of MexAB-

OprM efflux pump is probable offenders **[9]**. Nevertheless, the difference is paramount: VIM-producing strains disrupt nearly all 2 -lactams, meanwhile porin-deficient ones can still be susceptible to cephalosporins or such a combination as ceftolozane-tazobactam. Our data **(Table 3)** confirm that VIM-positive strains are much more resistant to ceftazidime (100%) and cefepime (97.1) in comparison to VIM-negative ones, which support the idea of the broad-spectrum hydrolytic activity of the enzyme. Moreover, our statistical analysis indicated that there is a very strong correlation between blaVIM carriage and non- beta lactam classes resistance. VIM positive isolates were much more resistant to Gentamicin (85.7% vs 37.5, $p<0.001$) and Ciprofloxacin (88.6% vs 56.3, $p<0.001$). The genetic background of the bla_{VIM} gene is the best explanation of this phenomenon. It is nearly solely located in Class 1 integrons which are gene capture systems able to store gene cassettes encoding aminoglycoside modifying enzymes (AMEs) and quinolone resistance genes **[10]**. The result of this genetic capitalism is the development of MDR and XDR phenotypes which result in providing clinicians with virtually no choices. Of particular concern is the failure of new combinations of novel -lactam/ -lactamase inhibitor (BL/BLIs) to counter the producers of MBL. Such agents as ceftazidime-avibactam and ceftolozane-tazobactam that are effective against KPC and OXA-48 producers are inactive against VIM metallo-enzymes **[11]**. As a result, VIM-producing *P. aeruginosa* has often required the administration of cefiderocol which is a newer siderophore cephalosporin which is resistant to MBLs, or the re-introduction of older, more toxic agents such as colistin **[12]**. Our investigation revealed that 98.3% were susceptible to colistin indicating that it is a suitable last-resort treatment but its nephrotoxicity is restrictive. New recommendations provided by the Infectious Diseases Society of America (IDSA) underline the role of molecular testing in informing therapy in CRPA **[13]**. We can substantiate this recommendation. The problem of relying only on the phenotypic susceptibility patterns can postpone proper treatment. An example of this is Aztreonam which is theoretically resistant to MBLs, 68.6% resistance was observed in VIM-positive strains. This can probably be explained by the fact that other enzymes (such as ESBLs or AmpC) also hydrolyze monobactams, so treatment should be combined (*e.g.*, Aztreonam with Avibactam) and not monotherapy **[14-17]**. It is notable as well that *P. aeruginosa* is environmentally persistent. The case is high in the ICU environment (55%), which indicates that there could be reservoirs of the hospital setting (sinks, ventilators) where the clonal progression of VIM-producing strains occurs **[18]**.

Conclusion:

bla_{VIM} drives carbapenem and multi-drug resistance in *P. aeruginosa*, rendering standard empirical treatments ineffective. Therefore, regular molecular surveillance is required to guide targeted therapies and proper antibiotic stewardship.

Limitations:

This study has limitations. The study is a single-center study and this can be a limitation to generalizability. Multilocus Sequence Typing (MLST) was not done to identify clonal relatedness and no full integrin structures were sequenced to identify particular VIM variants (*e.g.,* VIM-1 or VIM-2). Whole-genome sequencing should be studied in the future to trace the dynamics of transmission of these high-risk clones.

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