Alqalam Journal of Medical and Applied Sciences. 2025;8(3):1487-1491
https://doi.org/10.54361/ajmas.258333

Original article

Phenotypic and Genotypic Screening for VIM and IMP-Type Metallo-β-Lactamases in *Acinetobacter baumannii* Isolates from Tripoli Hospitals

Mohamed Ahmed[1], Abdulmottaleb Zetrini[2], Murad Daddesh[3], Najib Sufya[4], Hend Shubar[4], Nada Algrew[5], Naseem Abu khutwa[5], Hamza Atiya[1], Abdulaziz Zorgani[6], Abdulla Bashein[6]

[1]*Department of Clinical Biochemistry, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya*
[2]*Department of Pharmaceutics, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya*
[3]*Ministry of Health of Libya, Tripoli, Libya*
[4]*Department of Microbiology, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya*
[5]*Libyan Biotechnology research center, Tripoli, Libya*
[6]*Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Tripoli, Tripoli, Libya*
Corresponding email. *moh.abdulaziz@uot.edu.ly*

Abstract
Acinetobacter baumannii is a major nosocomial pathogen, particularly in intensive care units (ICUs), due to its remarkable resistance to multiple antibiotics and its persistence in hospital environments. The occurrence of carbapenem-resistant A. baumannii, often driven by the metallo-β-lactamase genes such as blaIMP and blaVIM, results in a serious clinical threat. In this study, 119 A. baumannii samples were collected from different hospitals in Tripoli, Libya. Real-time PCR revealed that 29 (24.3%) isolates carry the blaIMP gene and 62 (52%) have the blaVIM gene. Phenotypic detection of carbapenemase production revealed that chromogenic media had the highest detection rate (74.7%), followed by the Modified Hodge Test (67%) and E-test (47%). Antimicrobial susceptibility testing showed 100% resistance to ertapenem, cefoxitin, ceftriaxone, ampicillin, and amoxicillin-clavulanate, with high resistance also observed against imipenem (88.2%), meropenem (88.2%), gentamycin (94.9%), ciprofloxacin (94%), and levofloxacin (92.4%). Colistin was the only antibiotic to which all A. baumannii isolates remained sensitive. These findings underscore the high burden of multidrug resistance and the widespread presence of blaVIM and blaIMP genes in A. baumannii isolates in Libya. Improved molecular surveillance, reliable detection methods, and improved antibiotic stewardship are urgently needed to control the spread of these resistant pathogens.
Keywords. Acinetobacter Baumannii, Metallo-B-Lactamase, Blaimp, Blavim.

Introduction

Acinetobacter baumannii (A. baumannii) is a Gram-negative pathogen and a leading cause of hospital-acquired infections, particularly in intensive care units (ICUs) [1]. Its clinical significance lies in its capacity to cause life-threatening conditions, including ventilator-associated pneumonia, bacteremia, urinary tract infections, and wound infections, especially among critically ill or immunocompromised patients [2, 3]. Due to its increasing prevalence and multidrug resistance (MDR), the World Health Organization has classified MDR A. baumannii as a "critical priority" pathogen that urgently requires the development of new therapeutic options [4].

The A. baumannii's ability to acquire resistance against multiple antibiotic classes, including aminoglycosides, cephalosporins, fluoroquinolones, and most critically, carbapenems, poses a major clinical challenge to effective treatments [5]. Carbapenems such as imipenem, meropenem, and ertapenem are often considered the last choice for Gram-negative infections. However, the extensive resistance of A. baumannii to these antibiotics has severely limited therapeutic choices in clinical practice [5]. A primary mechanism of carbapenem resistance in A. baumannii is the production of carbapenem-hydrolyzing β-lactamases, particularly metallo-β-lactamases (MBLs). Among these, the blaIMP (imipenemase) and blaVIM (Verona integron-encoded metallo-β-lactamase) genes are particularly significant, as they are associated with high-level resistance and can spread rapidly through horizontal gene transfer [6,7]. These resistance determinants are typically located on plasmids and integrons, facilitating their dissemination within hospital environments and across bacterial populations [8,9]. The prevalence of blaIMP and blaVIM varies geographically and between healthcare institutions. Studies have shown a concerning increase in MBL-mediated resistance among A. baumannii strains in the Mediterranean region and parts of North Africa [10]. In Libya, the absence of localized data on the distribution of blaIMP and blaVIM genes hinders the implementation of targeted infection control measures and antimicrobial stewardship programs. Accurate phenotypic detection of carbapenemase-producing organisms is essential not only for guiding treatment decisions but also for tracking the epidemiology of resistance [11]. Common phenotypic methods include the E-test (gradient diffusion), the Modified Hodge Test (MHT), and chromogenic media-based assays. However, the diagnostic accuracy of these methods can vary widely in terms of sensitivity and specificity, and comparative evaluations from North African healthcare settings are lacking [12]. This study aimed to determine the prevalence of the blaIMP and blaVIM genes in A. baumannii isolates from ICU patients at different hospitals in Tripoli, Libya. In addition, the antimicrobial resistance profiles of

Received*: 20-05-2025 -* ***Accepted****: 17-07-2025 -* ***Published****: 23-07-2025*

these isolates were evaluated against several antibiotics. Our findings provide critical insights into the molecular epidemiology and diagnostic challenges associated with carbapenem-resistant A. baumannii in high-risk clinical settings.

Methods

Sample collections from patients

In this study, samples were collected from 119 patients (43 females and 76 males) from different hospitals located in Tripoli (Tripoli Medical Centre, Tripoli Pediatric Hospital, Burn and Plastic Surgery Hospital, and Tripoli Central Hospital). The age of the patients ranged from newborns to the elderly.

Isolation and identification of Acinetobacter baumannii *using conventional and automated microbiological techniques*

The collected samples were cultured on MacConkey agar within an hour of collection and incubated at 37 °C for 24 h to allow the colonies to form. The Presumptive identification of Acinetobacter baumannii was based on colony morphology; the formed colonies appeared smooth and sometimes mucoid with pale yellow to white, grayish colour. The Acinetobacter baumannii was further confirmed by Gram-stain, catalase test, oxidase test, and triple sugar iron test. A. baumannii was further identified using the BD Phoenix™ Automated Microbiology System with NMIC/ID panels, following the manufacturer's instructions. Ther Isolates were stored at −70°C for further analysis.

Molecular detection of blaVIM and blaIMP using Polymerase Chain Reaction (PCR):

Molecular identification of blaVIM and blaIMP genes was carried out using using primers *bla*-IMP-F (5′-CGATCTATCCCCACGTATGC-3′) *bla*-IMP-R (5′-CCACCGAATAATATTTTCCTTTCA-3′). Real-time PCR was used with already known primers, and Eva Green Real-time PCR Kit from

Antimicrobial susceptibility testing (AST)

The BD Phoenix Gram-negative antimicrobial susceptibility testing card was used to investigate the susceptibility of A. baumannii to several antimicrobial agents. The AST results for antimicrobials were classified as sensitive (S) or resistant (R) and are interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria.

Modified Hodge Test

This test was conducted to detect carbapenemase production in Gram-negative bacteria. A suspension of a carbapenem-susceptible strain was prepared, diluted, and evenly swabbed to create a lawn of growth on a Mueller-Hinton agar plate. A 10µg meropenem or ertapenem disk was placed at the center of the plate. The test organism was then streaked in a straight line from the antibiotic disk to the edge of the plate. The plate was incubated at 37°C for 24 hours. A positive result is indicated by visible growth of the test organism along the streak line toward the carbapenem disk, while a negative result shows no such increase.

Chromatic CRE

A chromogenic screening medium was used for the detection of carbapenem-resistant Enterobacteriaceae and non-fermenting Gram-negative bacilli. Following incubation, colony morphology and coloration were observed and interpreted according to the manufacturer’s instructions.

E-test (Epsilometer)

Phenotypic detection of VIM-type metallo-β-lactamases was carried out using E-test MBL strips (Liofilchem, Italy), which contain gradients of meropenem and meropenem-EDTA (MRP/MRD). The minimum inhibitory concentration (MIC) ratio was determined on Mueller-Hinton agar (MHA) according to the manufacturer's instructions (Epsilometer assay). All bacterial isolates were screened and interpreted based on CLSI guidelines. Each E-test was performed in triplicate, and the mean MIC value was used for data analysis.

Results

IMP and VIM genes identification in A. baumannii bacteria using real-time PCR

119 samples of A. baumannii bacteria isolated from wounds of hospitalized patients in the intensive care unit of the burn plastic surgery hospital located in Tripoli were screened for the presence of IMP and VIM genes using a PCR test. The PCR results showed that 29 samples (24.3%) out of 119 carry the IMP gene (Table 1). While the VIM gene was found in 62 (52%) samples out of 119 (Table 2).

Received*: 20-05-2025 -* ***Accepted****: 17-07-2025 -* ***Published****: 23-07-2025*

Table 1. PCR results for detecting the IMP gene

Total number of samples collected	Number of positive samples & (%)	Number of negative samples & (%)
119	29 (24.3%)	90 (75.7%)

Table 2. PCR results for detecting the VIM gene

Total number of samples collected	Number of positive samples & (%)	Number of negative samples & (%)
119	62 (52%)	57 (48%)

Phenotypic detection of carbapenemase-producing* A baumannii *using different methods

A total of 119 Acinetobacter baumannii clinical isolates were tested for carbapenemase production using different phenotypic methods: E-test, Modified Hodge Test (MHT), and chromogenic media (Table 3). Carbapenemase production was identified in 56 isolates (47%) using the E-test, in 80 isolates (67%) using the Modified Hodge Test, and in 89 isolates (74.7%) using chromogenic media. These findings indicate that chromogenic media yielded the highest detection rate, followed by the MHT, while the E-test demonstrated the lowest sensitivity among the three. The results are shown in Table 3.

Table 3. Phenotypic detection of carbapenemase-producing A. baumannii using different methods

A. baumannii Samples	E-Test (N, %)	MHT (N, %)	Chromogen media (N, %)
Numbers (%)	56 (47%)	80 (67%)	89 (74.7%)

Antibiotic resistance patterns of A. baumannii against several antibiotics

Antibiotic resistance was investigated using the BD Phoenix Gram-negative antimicrobial susceptibility testing card. Table 4 summarizes the resistance of A. baumannii against different antibiotics. For the carbapenem class of antibiotics, out of 119 A. baumannii collected from patients, 119 (100%) were found resistant to ertapenem, and 105 (88.2%) were found resistant to both imipenem and meropenem. For cephalosporins, cefoxitin and ceftriaxone were found to be resistant in all 119 (100%) samples, while 119, 113 (94.9%) samples were resistant to ceftazidime and cefepime.

For aminoglycosides, out of 119 samples, 113 (94%) showed resistance against gentamycin, while 95 (79.8%) were resistant to amikacin. In addition, colistin antibiotic, which belongs to the polymyxin group, showed sensitivity against all samples 119 (100%). 112 (94%) and 110 (92.4%) samples were resistant to ciprofloxacin and levofloxacin, respectively. For the beta-lactam class, Amoxicillin-clavulanate and Ampicillin were both 100% resistant. 103 (86.5%) and 114 (95.7%) of the total 119 samples were resistant against piperacillin-tazobactam and Aztreonam, respectively. Trimethoprim-sulfamethoxazole, which belongs to the antifolate class of antibiotics, showed 63% resistance. Among all these antibiotics, colistin, Trimethoprim-sulfamethoxazole, and amikacin showed the lowest resistance against A. baumannii bacteria.

Table 4: Antibiotic resistance patterns of A. baumannii against several antibiotics.

Class of antibiotics	Name of the Antibiotics	Number of resistant isolates (%)	Number of sensitive isolates (%)
Carbapenems	Ertapenem Imipenem Meropenem	119 (100 %) 105 (88.2 %) 105 (88.2 %)	0 (0 %) 14 (11.8%) 14 (11.8 %)
cephalosporins	Cefoxitin Ceftriaxone Ceftazidime Cefepime	119 (100 %) 119 (100%) 113 (94.9 %) 113 (94.9 %)	0 (0 %) 0 (0 %) 6 (5.1%) 6 (5.1%)
aminoglycosides	Gentamycin Amikacin	113 (94.9%) 95 (79.8 %)	6 (5.1 %) 24 (20.2 %)
polymyxin	Colistin	0 (0%)	119 (100%)
Fluoroquinolone	Ciprofloxacin Levofloxacin	112 (94%) 110 (92.4%)	7 (6%) 9 (7.6%)
Beta-lactam	Amoxicillin-clavulanate (Piperacillin-tazobactam) Aztreonam Ampicillin	119 (100%) 103 (86.5%) 114 (95.7%) 119 (100%)	0 (0%) 16 (13.5%) 5 (4.3%) 0 (0%)
Antifolate	Trimethoprim-sulfamethoxazole	75 (63%)	44 (37%)

Received*: 20-05-2025 -* ***Accepted****: 17-07-2025 -* ***Published****: 23-07-2025*

Discussion

The emergence and rapid spread of MDR Acinetobacter baumannii, particularly in ICUs, represents a major public health challenge. Our study aimed to investigate the prevalence of blaIMP and blaVIM metallo-β-lactamase (MBL) genes, assess antibiotic resistance profiles, and evaluate phenotypic methods for detecting carbapenemase-producing A. baumannii in a cohort of isolates from ICU patients in different hospitals in Tripoli, Libya.

The molecular findings demonstrated that blaVIM was more prevalent than blaIMP among the investigated isolates, with detection rates of 52% and 24.3%, respectively. This aligns with previous reports from similar healthcare settings, where blaVIM has emerged as the dominant MBL gene associated with carbapenem resistance in A. baumannii [13]. The presence of these genes in over half of the isolates highlights the significant role of MBLs in the dissemination of resistance and suggests active horizontal gene transfer within hospital environments.

Phenotypic detection methods showed variable sensitivity in identifying carbapenemase-producing A. baumannii. Chromogenic media demonstrated the highest detection rate (74.7%), followed by the Modified Hodge Test (67%) and E-test (47%). These findings suggest that chromogenic media may serve as a more reliable and rapid tool for routine screening of carbapenemase production in clinical laboratories, potentially aiding in early detection and infection control. The relatively low sensitivity of the E-test highlights the need for cautious interpretation when used in isolation [14].

Antibiotic susceptibility testing revealed alarming levels of resistance across most tested antibiotics. All isolates (100%) exhibited resistance to ertapenem, cefoxitin, ceftriaxone, ampicillin, and amoxicillin-clavulanate. Resistance to other critical antibiotics, such as imipenem and meropenem, was also high (88.2%), confirming that carbapenems are becoming increasingly ineffective in treating A. baumannii infections. The near-complete resistance to cephalosporins and aminoglycosides further limits therapeutic options. Notably, colistin remained fully effective against all isolates, consistent with its continued efficacy as a last-resort agent [15]. However, reliance on colistin raises concerns regarding nephrotoxicity and the potential emergence of colistin-resistant strains.

Among the non-carbapenem antibiotics, amikacin (79.8% resistance) and trimethoprim-sulfamethoxazole (63% resistance) showed relatively lower resistance rates, suggesting they may still have limited clinical utility in certain contexts. Nonetheless, the high overall MDR observed in this study underscores the need for strong antibiotic stewardship and the development of novel treatment strategies.

Collectively, our data reflect a critical antimicrobial resistance situation in ICU settings and reinforce the importance of integrating molecular and phenotypic methods for the surveillance of resistance mechanisms. The detection of high rates of blaVIM and blaIMP genes and the near-universal resistance to key antimicrobial classes should prompt urgent interventions, including enhanced infection control measures, genomic surveillance, and the rational use of antimicrobials.

Conclusion

This study highlights the high prevalence of multidrug-resistant Acinetobacter baumannii among ICU patients in four hospitals in Tripoli, Libya, with particularly alarming resistance to carbapenems and other major antibiotic classes. The widespread presence of blaVIM and blaIMP genes underscores the significant role of metallo-β-lactamases in mediating resistance and contributing to the therapeutic challenges posed by these pathogens. Among the phenotypic methods evaluated, chromogenic media demonstrated superior sensitivity for detecting carbapenemase-producing isolates and may serve as a valuable diagnostic tool in clinical microbiology laboratories. These findings emphasize the urgent need for continuous molecular surveillance, robust infection control practices, and limiting the use of antibiotics to limit the spread of resistant A. baumannii strains and preserve the effectiveness of last-resort therapies such as colistin.

Conflict of interest

The authors have no conflict of interest to declare.

References

1. Rahman MH, Siddique AB, Zihadi MAH, et al. Prevalence and molecular characterization of multi-drug and extreme drug resistant Escherichia coli in companion animals in Bangladesh. Sci Rep. 2025;15:16419.
2. Citron DM, Ostovari MI, Karlsson A, et al. Evaluation of the E test for susceptibility testing of anaerobic bacteria. J Clin Microbiol. 1991;29(10):2197-203.
3. Karakonstantis S, Kritsotakis EI, Gikas A. Treatment options for K. pneumoniae, P. aeruginosa and A. baumannii co-resistant to carbapenems, aminoglycosides, polymyxins and tigecycline: an approach based on the mechanisms of resistance to carbapenems. Infection. 2020;48(6):835-51.
4. Aurilio C, Sansone P, Barbarisi M, et al. Mechanisms of action of carbapenem resistance. Antibiotics. 2022;11(4):421.
5. Castañeda-Barba S, Top EM, Stalder T. Plasmids, a molecular cornerstone of antimicrobial resistance in the One Health era. Nat Rev Microbiol. 2024;22(1):18-32.

Received: *20-05-2025* - ***Accepted***: *17-07-2025* - ***Published***: *23-07-2025*

6. Hidalgo-Tenorio C, Bou G, Oliver A, et al. The Challenge of Treating Infections Caused by Metallo-β-Lactamase–Producing Gram-Negative Bacteria: A Narrative Review. Clin Microbiol Rev. 2024;37(1):e00098-23.
7. Nguyen M, Joshi SG. Carbapenem resistance in Acinetobacter baumannii, and their importance in hospital-acquired infections: a scientific review. J Appl Microbiol. 2021;131(6):2715-38.
8. Mondal AH, Khare K, Saxena P, et al. A review on colistin resistance: an antibiotic of last resort. Front Microbiol. 2024;12:772.
9. Tavares RD, Tacão M, Henriques I. Integrons are key players in the spread of beta-lactamase-encoding genes. Sci Total Environ. 2024;857(Pt 3):159421.
10. Caliskan-Aydogan O, Alocilja EC. A review of carbapenem resistance in Enterobacterales and its detection techniques. Microorganisms. 2023;11(6):1491.
11. Falagas ME, Kontogiannis DS, Zidrou M, et al. Global Epidemiology and Antimicrobial Resistance of Metallo-β-Lactamase (MBL)-Producing Acinetobacter Clinical Isolates: A Systematic Review. Antibiotics. 2025;14(5):557.
12. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev. 2008;21(3):538-82.
13. Garnacho-Montero J, Amaya-Villar R. Multiresistant Acinetobacter baumannii infections: epidemiology and management. Curr Opin Infect Dis. 2010;23(4):332-9.
14. Kumar N, Kaur N, Kumar H, et al. Selection of a Phenotypic Method for Detecting Carbapenemase-Producing Organisms: A Dilemma for Resource-Limited Settings. J Glob Antimicrob Resist. 2025;30:1-11.
15. Foglia F, Ambrosino A, Bashir S, et al. Prevalence of Acinetobacter baumannii Multidrug Resistance in University Hospital Environment. Antibiotics. 2025;14(5):490.

Received*: 20-05-2025 -* ***Accepted****: 17-07-2025 -* ***Published****: 23-07-2025*