Clin. Lab. 2026;72:XXX-XXX
©Copyright

ORIGINAL ARTICLE

Antimicrobial Resistance and Virulence Profiles of *Acinetobacter baumannii* in Pediatric Healthcare-Associated Bacteremia

Mohamed Mefreh [1], Maysaa E. S. Zaki [1], Mona Abdelaltif [2], Mona A. E.-H. A. El-Raouf [3], Ahmed H. El-Nagdy [4], Nadia G. Elrefaei [5], Sara G. Eissa [1]

[1] *Clinical Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt*
[2] *Pediatrics Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt*
[3] *Medical Microbiology and Immunology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt*
[4] *Microbiology Department, Faculty of Dentistry, Horus University, Damietta El Gadeeda, Egypt*
[5] *Vice Deanship for Academic Affairs, College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia*

ABSTRACT

Background: **Several factors play a role in the pathogenesis of *Acinetobacter baumannii (A. baumannii*). This study aimed to determine the virulence genes of *A. baumannii* isolated from healthcare-associated bacteremia (HAB) in pediatric patients, as well as their biofilm-forming ability and resistance to carbapenem antibiotics.**
Methods: **One hundred isolates of *A. baumannii* from healthcare-associated bacteremia in children, collected between January 2024 and January 2025, were subjected to biochemical identification using the API 20NE system, and confirmation of *A. baumannii* was performed by PCR targeting the *blaOXA-51-like* gene. Biofilm formation determination was quantified using the standard microtiter plate assay with crystal violet staining. Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar. Genetic studies using the polymerase chain reaction (PCR) were performed to detect virulence-associated genes, including *bap*, *surA1*, *pld*, *csuE*, *ompA*, *kps*, and iron acquisition genes (*basD*, *entE*, and *tonB*) [17-22]. Screening for carbapenemase genes (*blaOXA-23*, *blaOXA-24/40*, *blaOXA-58*, *blaNDM*) was also conducted.**
Results: ***A. baumannii*** **was able to form strong biofilm in 20%, intermediate biofilm in 30%, and weak biofilm in 25% of the isolates. All *A. baumannii* isolates contained the bap and surA1 genes (100%), the pld gene (84%), and the basD gene (79%).**
Conclusions: **The significant presence of *bap* and *surA1* genes among all imipenem-resistant and biofilm-forming isolates highlights their central role in enhancing both virulence and antimicrobial resistance. Moreover, there are significant correlations between imipenem resistance, *omp33-36*, and *blaOXA-40*, emphasizing the combined contribution of altered membrane permeability and carbapenemase activity to resistance phenotypes. The association of biofilm formation with *blaOXA-23* and *basD* genes further reflects a multifactorial resistance strategy.**
(Clin. Lab. 2026;72:xx-xx. DOI: 10.7754/Clin.Lab.2025.250964)

Correspondence:
Prof. Maysaa El Sayed Zaki
Professor of Clinical Pathology
Faculty of Medicine
Mansoura University
Mansoura
Egypt
Email: may_s@mans.edu.eg

Manuscript accepted November 3, 2025

KEYWORDS

A. baumannii, carbapenem resistance, biofilm formation, virulence genes

INTRODUCTION

Acinetobacter baumannii (*A. baumannii*) is an opportunistic Gram-negative pathogen responsible for a broad

spectrum of healthcare-associated infections, including bacteremia, ventilator-associated pneumonia, urinary tract infections, and wound infections [1,2]. Its clinical importance stems from its remarkable ability to acquire multidrug resistance (MDR), particularly to carbapenems, which are often the last line of effective therapy [3-7]. The World Health Organization has identified carbapenem-resistant *A. baumannii* as a critical priority pathogen, highlighting its global threat to public health and the urgent need for the development of new antimicrobials [8]. This designation reflects its high prevalence in intensive care units, association with prolonged hospital stays, and contribution to increased morbidity and mortality [9-11].

In addition to antimicrobial resistance, *A. baumannii* employs multiple virulence factors that enhance persistence and pathogenicity, including biofilm formation, capsular polysaccharides, outer membrane proteins, phospholipases, and iron acquisition systems [12-22]. Biofilm formation plays a pivotal role in survival within hospital environments, colonization of medical devices, and protection against host immune responses and antibiotics [12-16]. Virulence genes, such as bap, surA1, pld, and basD, contribute to adhesion, biofilm maturation, membrane disruption, and iron acquisition, thereby enhancing persistence and pathogenicity [17-20]. Additionally, carbapenemase genes such as blaOXA-23 and blaOXA-40 are frequently detected in multidrug-resistant strains, linking resistance mechanisms with virulence traits [21-23]. The interplay between these genetic determinants underscores the multifactorial strategies employed by *A. baumannii* to thrive in hostile clinical environments and complicate treatment outcomes [24]. Carbapenem resistance in *A. baumannii* is primarily mediated by carbapenem-hydrolyzing class D β-lactamases (CHDLs), notably OXA-type enzymes, and occasionally by metallo-β-lactamases such as NDM [23-28]. Additional mechanisms, including porin modifications such as CarO loss, further enhance resistance [29].

Despite numerous reports worldwide, there is a lack of data on the combined profiles of resistance, biofilm formation, and virulence determinants in pediatric healthcare-associated bacteremia caused by *A. baumannii*. Therefore, this study was designed to characterize antimicrobial resistance, biofilm formation, and virulence genes and assess their potential interrelationships in pediatric isolates from a tertiary care hospital in Egypt.

MATERIALS AND METHODS

Methods

This cross-sectional study included 100 non-duplicate *A. baumannii* isolates recovered from blood cultures of pediatric patients with healthcare-associated bacteremia at Mansoura University Children's Hospital, Egypt, between January 2024 and January 2025. Healthcare-associated infections (HAIs) were defined according to CDC criteria. Ethical approval was obtained from the Institutional Review Board of the Faculty of Medicine, Mansoura University.

Blood culture collection and processing

Venous blood samples were collected aseptically from pediatric patients using standard sterile technique. The venipuncture site was disinfected with 2% chlorhexidine in 70% isopropyl alcohol, and 1 - 4 mL of blood was drawn and immediately inoculated into BACT/ALERT® PF Plus pediatric bottles (bioMérieux, France). The inoculated bottles were loaded into the BACT/ALERT® 3D automated blood culture system (bioMérieux, France) and incubated at 35 - 37°C for up to 5 days or until a positive signal was detected.

Upon a positive signal, an aliquot was aseptically withdrawn from the flagged bottle and subcultured onto the following media: Blood agar base with 5% sheep blood (Oxoid™, UK), MacConkey Agar (Oxoid™, UK), and CHROMagar™ Acinetobacter (CHROMagar, France) for selective isolation of *Acinetobacter baumannii*. Plates were incubated aerobically at 35 - 37°C for 18 - 24 hours.

Bacterial identification

Initial identification of isolates was based on colony morphology, Gram staining, oxidase testing, and the API 20NE system (bioMérieux, France). Confirmation of *A. baumannii* was performed by PCR targeting the *blaOXA-51-like* gene [23].

Antimicrobial susceptibility testing

Antimicrobial susceptibility was assessed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, and the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [30]. Multidrug resistance (MDR) was considered non-susceptibility to at least one agent in three or more antimicrobial categories.

Biofilm assay

Biofilm formation was quantified using the standard microtiter plate assay with crystal violet staining [12,13]. Biofilm production was classified as negative, weak, moderate, or strong based on OD570 measurements. *A. baumannii* ATCC 19606 was used as a reference control strain.

Molecular detection of virulence and resistance genes

PCR assays were performed to detect virulence-associated genes, including *bap*, *surA1*, *pld*, *csuE*, *ompA*, *kps*, and iron acquisition genes (*basD*, *entE*, *tonB*) [17-22]. Screening for carbapenemase genes (*blaOXA-23*, *blaOXA-24/40*, *blaOXA-58*, *blaNDM*) was also conducted [23-25].

Statistical analysis

Data were analyzed using SPP24. Numerical data were represented as means ± SDs, while qualitative data were

Table 1. Genes, primer sequences, and annealing temperatures.

Gene	Primer sequence 5′→3′	Annealing temperature (°C)	Reference
16sRNA	F: 5 -CAGCTCGTGTCGTGAGATGT-3 R: 5 -CGTAAGGGCCATGATGACTT-3	55	[13]
blaOXA-	F: 5 -TAATGCTTTGATCGG CCT TG-3 R: 5 -TGGATTGCACTTCATCTTGG-3	52	[13]
bap	F: AGTTAAAGAAGGGCAAGAAG R: GGAGCACCACCTAACTGA	58	[17]
surA1	F: CAATTGGTAGCTGGCGATCA R: TTAGGCGGGACTCAGCTTTT 51.8	58	[18]
basD	F: CTCTTGCATGGCAACACCAC R: CCAACGAGACCGCTTATGGT	60	[19]
bauA	F: TGGCAAGGTGAAAATGCACG R: GCCGCATATGCCATCAACTG	60	[20]
pld	F: CCGTCAATTACGCCAAGCTG R: CTGACGCTACCTGACGGTTT	60	[21]
omp33-36	F: ATTAGCCATGACCGGTGCTC R: CCACCCCAAACATGGTCGTA	60	[22]
blaOXA-40	F: GGTTAGTTGGCCCCCTTAA R: AGTTGAGCGAAAAGGGGATT	58	[23]
blaOXA-23	F: GATCGGATTGGAGAACCAGA R: ATTTCTGACCGCATTTCCAT	58	[23]

Table 2. Antibiotic resistance rates of *A. baumannii* isolates.

Antibiotics	No.	(%)
Doxycycline	81	81
Trimethoprim/Sulfamethoxazole	42	42
Imipenem	74	74
Meropenem	74	74
Amikacin	58	58
Gentamicin	51	51
Tobramycin	72	72
Ciprofloxacin	55	55
Levofloxacin	29	29

Table 3. Frequency of virulence genes in *A. baumannii* isolates.

Genes	Frequency %
Bap	100
surA1	100
basD	79
bauA	19
pld	84
omp33-36	10
blaOXA-40	74
blaOXA-23	55

Table 4. Association between MDR and biofilm formation and virulence genes.

	MDR (n = 66)		Biofilm formation (n = 75)		p chi-square
	No.	%	No.	%	
Bap gene	66	100	75	100	p < 0.001 *
surA1	66	100	75	100	p < 0.001 *
basD	54	81.8	63	84	p * = 0.033
	0.44				
bauA	10	15.15	11	14.7	p = 0.056
	0.2				
Pld	54	81.8	61	81.3	p = 0.21
	0.57				
omp33-36	6	9.1	8	10.7	p = 0.7
	0.73				
blaOXA-40	52	78.8	56	74.7	p = 0.8
	0.15				
blaOXA-23	39	59.1	46	61.3	p = 0.03
	0.3				

*** p is significant.**

represented as numbers and percentages. Pearson's chi-squared test was used to compare qualitative data, and the significance level was set at $p < 0.05$. Also, the Kappa test was used to establish the agreement between qualitative data.

RESULTS

This study included 100 *A. baumannii* isolates obtained from 52 (52%) males and 48 (48%) females with bacteremia, with a mean age of 48.9 ± 11.5 years (data not shown). *A. baumannii* isolates demonstrated high resistance to doxycycline (81%), imipenem and meropenem (74%) each, and tobramycin (72%). The lowest resistances were measured for levofloxacin (29%) and trimethoprim/sulfamethoxazole (42%). MDR was reported among 66 isolates (66%), as shown in Table 2.

Seventy-five isolates of *A. baumannii* showed the capacity to form biofilm, with strong biofilm in 20%, intermediate biofilm in 30%, and weak biofilm in 25% of the isolates (data not shown).

All *A. baumannii* isolates harbored the bap and surA1 genes (100%), 84% possessed the pld gene, 79% had the basD gene, and blaOXA-40 and blaOXA-23 were detected in 74% and 55% of the isolates, respectively. The least detected genes were omp33-36 (10%) and bauA (19%), as shown in Table 3.

All MDR isolates harbored the bap and surA1 genes (100%), each with a strong and significant association, as determined by the chi-squared test ($p < 0.001$). At the same time, other genes had statistically insignificant correlation between MDR and the presence of genes (basD-p = 0.44, bauA-p = 0.2, Pld-p = 0.57, omp33-36-p = 0.73, blaOXA-40-p = 0.15, blaOXA-23-p = 0.3). Moreover, there was a substantial agreement ($p < 0.001$) between the biofilm-forming capacity and the presence of bap and surA1 genes, as well as a significant association with the blaOXA-23 gene (p = 0.03) and the basD gene (p = 0.033), while other genes had insignificant association with biofilm (bauA-p = 0.056, Pld-p = 0.12, omp33-36-p = 0.7, blaOXA-40-p = 0.8), as shown in Table 4.

A significant association was found between biofilm formation and MDR ($p < 0.001$). All isolates with imipenem resistance carried both bap and surA1 genes, with a substantial agreement between the presence of omp33-36 and blaOXA-40 genes and imipenem resistance (p = 0.01, $p < 0.001$, respectively) - data not shown.

DISCUSSION

In this study, *A. baumannii* isolates from pediatric healthcare-associated bacteremia exhibited high levels of antimicrobial resistance, particularly to carbapenems, consistent with global reports of MDR and XDR phenotypes [1-11]. Resistance rates in our isolates were similar to those reported in regional studies in Saudi Arabia and Jordan [8,9] but higher than those observed in earlier reports [10,11], reflecting the increasing selective pressure and clonal dissemination of resistant strains.

Biofilm formation was detected in most isolates, with strong producers predominating, in agreement with earlier studies [12-16]. Biofilm-producing strains were more frequently resistant to multiple antibiotics, supporting the established convergence of biofilm formation and antimicrobial resistance [13,14]. These findings emphasize the clinical challenges posed by biofilm-as-

sociated *A. baumannii* infections, particularly in bloodstream and device-related infections.

Virulence gene profiling demonstrated a high prevalence of bap, ompA, kps, and iron acquisition genes, underscoring the multifactorial mechanisms underlying persistence and pathogenicity [17-22]. The detection of surA1 and pld, both of which are associated with virulence and survival, further highlights their role in pediatric infections. The widespread occurrence of iron uptake systems such as basD and tonB reflects the critical role of iron acquisition in adaptation to the host environment [21,22].

Molecular screening revealed diverse carbapenemase genes, with blaOXA-23 being the most common, consistent with global and regional reports [23-27]. The occasional detection of blaNDM further underscores the emerging threat of metallo-β-lactamases [25]. The contribution of porin modifications, particularly CarO loss, as described elsewhere [29], may also play a role in resistance; however, this was not evaluated in our study. Taken together, these findings demonstrate the coexistence of extensive drug resistance, strong biofilm production, and multiple virulence determinants in pediatric *A. baumannii* isolates. The clinical implications include limited therapeutic options, prolonged hospital stays, and increased risk of treatment failure. These results highlight the urgent need for stringent infection control measures, continuous molecular surveillance, and stewardship programs tailored to pediatric populations to combat this pathogen.

Future research should focus on elucidating the molecular pathways linking biofilm formation and resistance mechanisms, as well as exploring novel therapeutic strategies, such as anti-biofilm agents, bacteriophage therapy, and inhibitors targeting OXA-type carbapenemases. In addition, large-scale genomic surveillance studies are warranted to monitor the evolution of resistance and virulence determinants in pediatric settings. Developing alternative treatment modalities, including immunotherapies or vaccines targeting conserved virulence factors, may offer promising approaches to reduce the morbidity and mortality associated with multidrug-resistant *A. baumannii*.

CONCLUSION

This study highlights the alarming burden of *A. baumannii* in pediatric healthcare-associated bacteremia, characterized by high multidrug and carbapenem resistance rates, robust biofilm-forming ability, and the widespread presence of key virulence genes. The significant associations between biofilm formation, *bap* and *surA1* genes, and resistance determinants such as *blaOXA-23* underscore the synergistic interplay between antimicrobial resistance and pathogenicity.

Data Availability Statement:
The data utilized to reinforce the study's conclusions were posted to Figshare at https://doi.org/10.6084/m9.figshare.29004464.v1.

Ethical Approval Statement:
Written consent was obtained from all individuals, and the study was approved by the Mansoura Faculty of Medicine´s Ethical Committee (R.25.05.3181) in Egypt. All the children's parents gave written informed consent.

Declaration of Generative AI in Scientific Writing:
An artificial intelligence tool (ChatGPT, GPT-5, Open AI) was used for text editing and formatting support. The authors reviewed and approved all modifications and are accountable for the manuscript’s content.

Declaration of Interest:
None.

References:

1. Lee CR, Lee JH, Park M, et al. Biology of Acinetobacter baumannii: pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. Front Cell Infect Microbiol 2017;7:55. (PMID: 28348979)
2. Antunes LCS, Visca P, Towner KJ. Acinetobacter baumannii: evolution of a global pathogen. Pathog Dis 2014;71(3):292-301. (PMID: 24376225)
3. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. J Infect Dis 2008;197(8): 1079-81. (PMID: 18419525)
4. Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 2018;18(3):318-27. (PMID: 29276051)
5. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev 2008;21 (3):538-82. (PMID: 18625687)
6. Kyriakidis I, Vasileiou E, Pana ZD, Tragiannidis A. Acinetobacter baumannii antibiotic resistance mechanisms. Pathogens 2021; 10(3):373. (PMID: 33808905)
7. Poirel L, Nordmann P. Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. Clin Microbiol Infect 2006;12(9):826-36. (PMID: 16882287)
8. Alharbi O, Al-Said HM, Ashgar SS, et al. Prevalence and antibiogram of Acinetobacter baumannii from 2013 to 2023 in Saudi Arabia. Antibiotics (Basel) 2025;14(3):274. (PMID: 40149085)
9. Al-Tamimi M, Albalawi H, Alkhawaldeh M, et al. Multidrug-resistant Acinetobacter baumannii in Jordan. Microorganisms 2022; 10(5):849. (PMID: 35630295)

10. Manchanda V, Sanchaita S, Singh N. Multidrug-resistant Acinetobacter. J Glob Infect Dis 2010;2(3):291-304. (PMID: 20927292)

11. Roca I, Espinal P, Vila-Farrés X, Vila J. The Acinetobacter baumannii oxymoron: commensal hospital dweller turned pan-drug-resistant menace. Front Microbiol 2012;3:148. (PMID: 22536199)

12. Longo F, Vuotto C, Donelli G. Biofilm formation in Acinetobacter baumannii. New Microbiol 2014;37(2):119-27. (PMID: 24858639)

13. Roy S, Chowdhury G, Mukhopadhyay AK, et al. Convergence of biofilm formation and antibiotic resistance in Acinetobacter baumannii infection. Front Med (Lausanne) 2022;9:793615. (PMID: 35402433)

14. Javadi K, Ghaemian P, Baziboron M, Pournajaf A. Investigating the Link Between Biofilm Formation and Antibiotic Resistance in Clinical Isolates of *Acinetobacter baumannii*. Int J Microbiol 2025;2025:1009049. (PMID: 39974278)

15. Rodríguez-Baño J, Martí S, Soto S, et al. Biofilm formation in Acinetobacter baumannii: associated features and clinical implycations. Clin Microbiol Infect 2008;14(3):276-8. (PMID: 18190568)

16. Zeighami H, Valadkhani F, Shapouri R, Samadi E, Haghi F. Virulence characteristics of multidrug resistant biofilm forming Acinetobacter baumannii isolated from intensive care unit patients. BMC Infect Dis 2019;19:629. (PMID: 31382976)

17. Loehfelm TW, Luke NR, Campagnari AA. Identification and characterization of an Acinetobacter baumannii biofilm-associated protein. J Bacteriol 2008;190(3):1036-44. (PMID: 18024522)

18. Russo TA, Luke NR, Beanan JM, et al. The K1 capsular polysaccharide of A. baumannii is a major virulence factor. Infect Immun 2010;78(9):3993-4000. (PMID: 20643860)

19. Liu D, Liu ZS, Hu P, et al. Characterization of SurA1 and its role in virulence and fitness. Vet Microbiol 2016;186:126-38. (PMID: 27016767)

20. Jacobs AC, Hood I, Boyd KL, et al. Inactivation of phospholipase D diminishes A. baumannii pathogenesis. Infect Immun 2010;78 (5):1952-62. (PMID: 20194595)

21. Gaddy JA, Arivett BA, McConnell MJ, López-Rojas R, Pachón J, Actis LA. Role of acinetobactin-mediated iron acquisition functions in the interaction of Acinetobacter baumannii strain ATCC 19606T with human lung epithelial cells, Galleria mellonella caterpillars, and mice. Infect Immun 2012;80(3):1015-24. (PMID: 22232188)

22. Gentile V, Frangipani E, Bonchi C, Minandri F, Runci F, Visca P. Iron and Acinetobacter baumannii biofilm formation. Pathogens 2014;3(3):704-19. (PMID: 25438019)

23. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of Acinetobacter baumannii by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. J Clin Microbiol 2006;44(8):2974-6. (PMID: 16891483)

24. Woodford N, Ellington MJ, Coelho JM, et al. Multiplex PCR for genes encoding OXA carbapenemases in Acinetobacter spp. Int J Antimicrob Agents 2006;27(4):351-3. (PMID: 16564159)

25. Rahbarnia L, Farajnia S, Khaneshi H, et al. Detection of blaOXA-23 and blaNDM-1 in clinical isolates of *A. baumannii* in Tabriz, north-west of Iran. Gene Rep 2019;18:100555. Available from: https://www.sciencedirect.com/science/article/abs/pii/S2452014419301979

26. Domingues R, Oliveira R, Silva S, et al. Molecular Detection of Carbapenemases in Acinetobacter baumannii Strains of Portugal and Association With Sequence Types, Capsular Types, and Virulence. Clin Ther 2024;46(12):e9-e15. (PMID: 39384436)

27. Iovleva A, Mustapha MM, Griffith MP, et al. Carbapenem-Resistant Acinetobacter baumannii in U.S. Hospitals: Diversification of Circulating Lineages and Antimicrobial Resistance. mBio 2022;13(2):e0275921. (PMID: 35239023)

28. Evans BA, Amyes SG. OXA beta-lactamases. Clin Microbiol Rev 2014;27(2):241-63. (PMID: 24696435)

29. Siroy A, Molle V, Lemaître-Guillier C, et al. Channel formation by CarO, the carbapenem resistance-associated outer membrane protein of Acinetobacter baumannii. Antimicrob Agents Chemother 2005;49(12):4876-83. (PMID: 16304161)

30. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. Wayne, PA: CLSI; 2020. Available from: https://clsi.org/shop/standards/m100/