



Significant Increase in *bla*_{NDM-1} *Acinetobacter baumannii* in a Tertiary Care Greek Hospital Driven by the Dissemination of Established Clones and the Emergence of Novel Lineages

Stylianos Xitsas [†], Christos-Georgios Gkoutinoudis [†], Amalia Arkouda and Efthymia Petinaki ^{*}

Faculty of Medicine, University of Thessaly, 41500 Larissa, Greece; stexit007@gmail.com (S.X.); gkoutinoudis@gmail.com (C.-G.G.); amaliaarkouda1990@gmail.com (A.A.)

^{*} Correspondence: petinaki@uth.gr

[†] These authors contributed equally to this work.

Abstract

This study aimed to investigate the marked increase in *bla*_{NDM-1}-positive *Acinetobacter baumannii* isolates (20% of all CRAB isolates) observed in a tertiary-care hospital in Central Greece during 2025. Thirty *bla*_{NDM-1}-producing carbapenem-resistant *A. baumannii* (CRAB) isolates were selected and subjected to whole-genome sequencing for molecular typing and characterization of antimicrobial resistance determinants and virulence-associated genes. The majority of the isolates (22/30) belonged to ST2^{Pasteur} (18 strains), ST85^{Pasteur} (2 strains) and ST2493^{Pasteur} (2 strains), all previously identified in 2023. The remaining eight strains belonged to newly detected STs, such as ST1^{Pasteur} (six strains) and ST2806^{Pasteur} (two strains). All strains carried the *bla*_{NDM-1} gene on the *Tn*7382. The high prevalence of *bla*_{NDM-1}-positive CRAB represents not only a local threat but also a national concern, as patients from our hospital are frequently transferred to other healthcare facilities or long-term care centers across the country, creating a substantial risk for further dissemination of these strains. Routine molecular screening for *bla*_{NDM-1}-positive CRAB may represent a valuable strategy for limiting further dissemination.

Keywords: *Acinetobacter baumannii*; antibiotic resistance; *bla*_{NDM-1}; carbapenem; Greece; molecular epidemiological study



Academic Editor: Tomislav S. Kostyanov

Received: 20 May 2026

Revised: 21 June 2026

Accepted: 25 June 2026

Published: 30 June 2026

Copyright: © 2026 by the authors.

Published by MDPI on behalf of the Hellenic Society for Microbiology.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Acinetobacter baumannii (AB) is considered a major public health threat worldwide [1]. It is a Gram-negative bacterium that has become notorious for its ability to survive in hospital environments and for its capacity to develop multi-drug resistance (MDR) [2]. To date, more than 90% of the clinical isolates are resistant to nearly all commonly used antibiotics, including carbapenems, which are the last-resort antimicrobial agents for MDR Gram-negative bacteria [3]. The main mechanism of carbapenem-resistant *A. baumannii* (CRAB) is the acquisition of carbapenem-hydrolyzing β -lactamases, either of class D (such as OXA-23, OXA-24, OXA-58, etc.) or class B (such as IMP, NDM, etc.), enhanced by intrinsic mechanisms including high-level expression of OXA-51-like enzymes, reduced permeability, and efflux pump overexpression [4–6]. The rate and the type of carbapenemases among CRAB vary between countries and continents [7]. In Greece, the first report of *bla*_{NDM-1}-positive CRAB was published in 2023, in a tertiary-care hospital of Central Greece (the University Hospital of Larissa, UHL) [7]. Four different STs have been characterized according to the Pasteur scheme (STs 160, 85, 2493 and 2), which carried the *bla*_{NDM-1} gene

on two transposons, either on *Tn125* (ST160) or on *Tn7382* (ST85, ST2493, ST2). After this finding, surveillance programs were organized by the Committee of Nosocomial Infections of UHL in order to limit the dissemination of these strains. However, despite the strict measures (hand hygiene, environmental cleaning, isolation measures), the rate of *bla*_{NDM-1}-positive CRAB increased markedly from 8% of all CRAB isolates in 2024 to 20% in 2025. The aim of this epidemiological study is the molecular characterization of representative *bla*_{NDM-1}-positive CRAB isolates, collected during 2025 from different wards of UHL, in order to clarify if this increase is due to the dissemination of the previously described clones or to the emergence of new ones.

2. Materials and Methods

2.1. Collection of the *bla*_{NDM-1} Positive CRAB

During 2025, a total of 217 CRAB, representing 95% of the total AB isolates, were collected from clinical samples sent to the microbiological department of UHL. Identification and susceptibility testing of the isolates were performed by the automated Vitek-2 system (BioMerieux, Marcy l'Ecole, France), according to the guidelines of the routine protocol of the Microbiological laboratory of the UHL. Determination of MIC to colistin was performed by the broth microdilution method (ComASP™ Colistin, Liofilchem, Roseto degli Abruzzi, Italy), according to EUCAST guidelines (www.eucast.org). All isolates with MIC values to imipenem or meropenem equal to or greater than 2 mg/L were tested for the presence of *bla*_{OXA-23}, *bla*_{OXA-58} and *bla*_{NDM-1} by polymerase chain reaction (PCR) [7]. According to the PCR preliminary results, forty-four isolates (20% of the total CRAB), all normally distributed during the study period, carried the *bla*_{NDM-1}. Of the aforementioned isolates, thirty of them, isolated from clinically significant samples (blood cultures and bronchoalveolar lavages), were selected for molecular analysis. The selection was based on ward and time of isolation and on the patients' medical records, with particular emphasis on previous hospitalization in long-term care facilities or travel abroad.

2.2. Whole Genome Sequencing of *bla*_{NDM-1} Positive CRAB

From each isolate, the DNA was extracted using the PSS magLEAD 12Gc automated system (BioServices, Stockholm, Sweden). DNA quantity and purity were assessed using both Nanodrop One and Qubit fluorometers (Thermo Fisher Scientific, Waltham, MA, USA). Genomic sequencing was performed on the DNBSEQ-G99 platform (MGI, Shenzhen, China) using the MGIEasy Fast FS Library Prep Set, MGIEasy Dual Barcode Circulization Kit, and the High-Throughput Sequencing Set (G99 SM FCL PE150), aiming for 4 million reads per sample. Before the assembly of the microbial genome of each sample, the MGI Pathogen Fast Identification (PFI) tool was used for the molecular identification of microorganisms. Then, raw reads were assembled using SPAdes (version 4.0.0), guided by the closest reference genome corresponding to the dominant species identified by the MGI Pathogen Fast Identification (PFI) tool. The assembled genomes were processed for MLST typing, presence of antimicrobial resistance genes and point mutations, and characterization of virulence factors using the online tools PubMLST, AmrProfiler, and Virulence Factor Data Base [8–11]. A blast analysis was carried out for the characterization of the genetic contexts of the *bla*_{NDM-1} gene.

2.3. Nucleotide Accession Numbers

Representative genomes from each ST of the *bla*_{NDM-1}-positive CRAB have been deposited in GenBank under the BioProject: PRJNA1467426.

3. Results

3.1. Antimicrobial Phenotypes and Multi-Locus Sequence Types (MLST)

All *bla*_{NDM-1}-positive *A. baumannii*, were carbapenem-resistant, with MIC values of imipenem and meropenem both exceeding 16 mg/L. Based on the EUCAST breakpoints, the rates of resistance to amikacin, ciprofloxacin, colistin, gentamicin, levofloxacin, piperacillin-tazobactam, tobramycin and trimethoprim-sulfamethoxazole were 96%, 100%, 80%, 93%, 100%, 100%, 90% and 100%, respectively.

For molecular typing, both the Pasteur and Oxford MLST schemes were used to achieve improved epidemiological discrimination. Consequently, each isolate was defined by the combined ST^{Pasteur} and ST^{Oxford} profiles. Certain isolates were assigned to two possible ST^{Oxford} sequence types due to the simultaneous presence of two distinct *gdhB* alleles [12].

Of the 30 isolates examined, 17 (56%) were identified as ST2^{Pasteur} and ST3341/425^{Oxford} (pattern 1). Six strains belonged to the ST1^{Pasteur} // ST1567/441 (pattern 2). The remaining seven strains were classified as follows: ST2^{Pasteur} // ST1816/195^{Oxford} (one, pattern 6), ST2806^{Pasteur} // ST3474^{Oxford} (two, pattern 3), ST2493^{Pasteur} // ST1955^{Oxford} (two, pattern 4), and ST85^{Pasteur} // ST1089^{Oxford} (two, pattern 5).

Comparison with data from our previous study demonstrated the emergence of three new sequence type combinations: ST1^{Pasteur} // ST1567/441^{Oxford}, ST2^{Pasteur} // ST1816/195^{Oxford}, and ST2806^{Pasteur} // ST3474^{Oxford}. The remaining STs corresponded to clones previously identified in 2023 [7].

3.2. Identification of Resistance Genes and Characterization of Point Mutation

WGS analysis revealed the molecular background of the strains tested. Twelve genes were associated with β -lactam resistance (*bla*_{NDM-1}, *bla*_{OXA-20}, *bla*_{OXA-23}, *bla*_{OXA-66}, *bla*_{OXA-69}, *bla*_{OXA-94}, *bla*_{TEM-1}, *bla*_{ADC-11}, *bla*_{ADC-73}, *bla*_{ADC-186}, *bla*_{ADC-223}, *bla*_{ADC-268}), with various combinations among the strains. The analysis also revealed the presence of six genes linked to aminoglycoside resistance (*aph*(3')-Ia, *aph*(3')-Ib, *aph*(3')-VI, *aph*(6)-Id, *ant*(3'')-IIa, *armA*), two genes conferring resistance to sulphonamides (*sul1*, *sul2*), two genes conferring resistance to macrolides (*mphE*, *msrE*), one gene conferring resistance to tetracyclines (*tetB*), and one gene conferring resistance to trimethoprim (*drfA40*). Resistance to quinolones was related to the mutations S81L in the *gyrA* gene and S84L in the *parC* gene (Table 1). Despite the high rate of resistance to colistin, no resistant determinants such as *mcr* genes or mutations were detected.

Table 1. Distribution of the strains into six patterns: different ST combinations based on Pasteur and Oxford schemes, with the respective resistant determinants' profile. The presence is indicated by the purple color.

Pattern	Number of Strains	MSLT Pasteur	MSLT Oxford	AMR Genes																		Point Mutations				
				<i>bla</i> NDM-1	<i>bla</i> OXA-20	<i>bla</i> OXA-23	<i>bla</i> OXA-66	<i>bla</i> OXA-69	<i>bla</i> OXA-94	<i>bla</i> TEM-1	<i>bla</i> ADC-11	<i>bla</i> ADC-73	<i>bla</i> ADC-186	<i>bla</i> ADC-223	<i>bla</i> ADC-268	<i>tet</i> (B)	<i>dfp</i> A40	<i>Sul</i> I	<i>mph</i> (E)	<i>msn</i> (E)	<i>aph</i> (3')-Ia	<i>aph</i> (3')-Ib	<i>aph</i> (3')-VI	<i>anh</i> (6)-Id	<i>art</i> (3'')IIa	<i>gyrA</i>
1	17	2	3341/425																						S81L	S84L
2	6	1	1567/441																						S81L	S84L
3	2	2806	3474																						S81L	S84L
4	2	2493	1955																						S81L	S84L
5	2	85	1089																						S81L	S84L
6	1	2	1816/195																						S81L	S84L

In addition, WGS analysis revealed the presence of various genes encoding virulence factors. These included genes for exotoxin production, immune system modulation and biofilm formation (*plc1*, *plc2*, *plcD*, *lpxA*, *lpxB*, *lpxC*, *lpxD*, *lpxL*, *lpxM*, *ompA*, *bap*, *adeG*, etc.).

The differences in the presence of virulent determinants between the STs are described in Table 2.

Table 2. Differences regarding the presence of virulent determinants between the various STs. The presence is indicated by the symbol +.

Pattern	MSLT Pasteur	MLST Oxford	<i>cusA</i>	<i>cusB</i>	<i>cusC</i>	<i>cusD</i>	<i>cusE</i>	<i>pgaD</i>
1	2	3341/425	+		+	+	+	
2	1	1567/441	+		+	+	+	
3	2806	3474	+		+	+	+	
4	2493	1955						
5	85	1089	+	+	+	+	+	
6	2	1816/195	+	+	+	+	+	+

3.3. Genetic Environment of *bla*_{NDM-1}

The *bla*_{NDM-1} gene was located on the transposon Tn7382 [13]. This transposon encompasses seven open reading frames, *cutA-tat-iso-ble- bla*_{NDM-1}-IS*Aba125-aph*(3′)-*VI*, bracketed by two direct copies of IS*Aba14* [7].

4. Discussion

The present study is a follow-up to our previous investigation conducted in 2023 and aimed to evaluate the factors associated with the significant increase in *bla*_{NDM-1}-positive CRAB observed in 2025. The majority of the isolates (17/30) belonged to ST2^{Pasteur} // ST3341/425^{Oxford}, which had also been detected in 2023 [7]. Our findings suggest that the persistence and dissemination of this lineage substantially contributed to the increase in *bla*_{NDM-1}-positive CRAB isolates observed during 2025. It is well established that ST2^{Pasteur} strains (International Clone II) possess a strong ability to produce biofilm, enhancing their capacity to adhere to medical devices and hospital environmental surfaces, thereby facilitating their rapid dissemination.

Nine isolates belonged to newly emerged sequence type combinations that had not been previously identified in our institution, such as ST1^{Pasteur} // ST1567/441^{Oxford}, ST2806^{Pasteur} // ST3474^{Oxford}, and ST2^{Pasteur} // ST1816/195^{Oxford}. Recently, *bla*_{NDM-1}-positive CRAB isolates belonging to ST1^{Pasteur} // ST231^{Oxford} and ST2^{Pasteur} // ST218^{Oxford} were reported in Spain [14]. However, although these Spanish isolates shared the same Pasteur STs as our strains, they differed in their Oxford STs. It is known that the typing according to the Pasteur scheme is widely used, since the ST1^{Pasteur}, ST2^{Pasteur}, and ST85^{Pasteur} represent the major CRAB lineages worldwide; however, the additional use of the Oxford scheme provides a more precise epidemiological classification, specifically among closely related strains.

To date, three transposons carrying *bla*_{NDM-1} have been identified worldwide: the *Tn125*, which is the most prevalent, as well as the *Tn7382* and the *Tn6924*. All isolates in our study harbored *Tn7382* in their genomes; this structure was previously detected on the chromosome of several clones that originated from Saudi Arabia (ST2, ST570), Portugal (ST85), China (ST164), Belgium (ST85), Spain (ST85), or the USA (ST570) [7]. Thus, our findings reinforce the fact that the spread of the *bla*_{NDM-1} gene in *A. baumannii* is not linked to a clonal spread but to the spread of a genetic structure.

Although CRAB remains among the three most common nosocomial pathogens in Greece, reports of NDM-1-producing strains remain limited. The increasing prevalence of *bla*_{NDM-1}-positive CRAB represents not only a local threat but also a national concern, as patients from our hospital are frequently transferred to other healthcare facilities or long-term care centers across the country, creating a substantial risk for further dissemination of

these strains. Screening of the patients for the detection of *bla*_{NDM-1}-positive CRAB may constitute an effective adjunctive measure to prevent further spread.

Author Contributions: Conceptualization, S.X. and E.P.; methodology, C.-G.G. and A.A.; writing—original draft preparation, S.X. and E.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The genomes of the *bla*_{NDM-1}-positive *A. baumannii* isolates have been deposited in GenBank under BioProject accession PRJNA1467426.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Peleg, A.Y.; Seifert, H.; Paterson, D.L. *Acinetobacter baumannii*: Emergence of a successful pathogen. *Clin. Microbiol. Rev.* **2008**, *21*, 538–582. [[CrossRef](#)] [[PubMed](#)]
2. Fournier, P.E.; Richet, H.; Weinstein, R.A. The epidemiology and control of *Acinetobacter baumannii* in health care facilities. *Clin. Infect. Dis.* **2006**, *42*, 692–699. [[CrossRef](#)] [[PubMed](#)]
3. Nowak, J.; Zander, E.; Stefanik, D.; Higgins, P.G.; Roca, I.; Vila, J.; McConnell, M.J.; Cisneros, J.M.; Seifert, H. High incidence of pandrug-resistant *Acinetobacter baumannii* isolates collected from patients with ventilator-associated pneumonia in Greece, Italy and Spain as part of the MagicBullet clinical trial. *J. Antimicrob. Chemother.* **2017**, *72*, 3277–3282. [[CrossRef](#)] [[PubMed](#)]
4. Nutman, A.; Temkin, E.; Wullfhart, L.; Schechner, V.; Schwaber, M.J.; Carmel, Y. *Acinetobacter baumannii* bloodstream infections: A nationwide study in Israel. *Microorganisms* **2023**, *11*, 2178. [[CrossRef](#)] [[PubMed](#)]
5. Poirel, L.; Nordmann, P. Carbapenem resistance in *Acinetobacter baumannii*: Mechanisms and epidemiology. *Clin. Microbiol. Inf.* **2006**, *12*, 826–836. [[CrossRef](#)]
6. Bonnin, R.A.; Poirel, L.; Nordmann, P. New Delhi metallo- β -lactamase-producing *Acinetobacter baumannii*: A novel paradigm for spreading antibiotic resistance genes. *Future Microbiol.* **2014**, *9*, 33–34. [[CrossRef](#)] [[PubMed](#)]
7. Tsilipounidaki, K.; Gkoutinoudis, C.G.; Florou, Z.; Fthenakis, G.C.; Miriagou, V.; Petinaki, E. The Molecular Characterization of *bla*_{NDM-1}-Positive *Acinetobacter baumannii* Isolated in Central Greece. *Microorganisms* **2023**, *11*, 2588. [[CrossRef](#)] [[PubMed](#)]
8. Bartual, S.G.; Seifert, H.; Hippler, C.; Luzon, M.A.; Wisplinghoff, H.; Rodríguez-Valera, F.J. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. *Clin. Microbiol.* **2005**, *43*, 4382–4390. [[CrossRef](#)]
9. Diancourt, L.; Passet, V.; Nemec, A.; Dijkshoorn, L.; Brisse, S. The population structure of *Acinetobacter baumannii*: Expanding multiresistant clones from an ancestral susceptible genetic pool. *PLoS ONE* **2010**, *5*, e10034. [[CrossRef](#)] [[PubMed](#)]
10. Skoulakis, A.; Kostoula, V.; Daniilidis, K.; Gkoutinoudis, C.G.; Petinaki, E.; Hatzigeorgiou, A.G. AmrProfiler: A comprehensive tool for identifying antimicrobial resistance genes and mutations across species. *Nucleic Acids Res.* **2025**, *53*, W20–W31. [[CrossRef](#)] [[PubMed](#)]
11. Chen, L.; Xiong, Z.; Sun, L.; Yang, J.; Jin, Q. VFDB 2012 update: Toward the genetic diversity and molecular evolution of bacterial virulence factors. *Nucleic Acids Res.* **2012**, *40*, D641–D645. [[CrossRef](#)] [[PubMed](#)]
12. Gaiarsa, S.; Batisti Biffignandi, G.; Esposito, E.P.; Castelli, M.; Jolley, K.A.; Brisse, S.; Sasser, D.; Zarrilli, R. Comparative Analysis of the Two *Acinetobacter baumannii* Multilocus Sequence Typing (MLST) Schemes. *Front. Microbiol.* **2019**, *10*, 930. [[CrossRef](#)] [[PubMed](#)]
13. Hamed, S.M.; Hussein, A.F.A.; Al-Agamy, M.H.; Radwan, H.H.; Zafer, M.M. Tn7382, a novel composite transposon harboring *bla*_{NDM-1} and *aphA6* in *Acinetobacter baumannii*. *J. Glob. Antimicrob. Resist.* **2022**, *30*, 414–417. [[CrossRef](#)] [[PubMed](#)]
14. Hidalgo, E.; Sotelo, J.; Pérez-Vázquez, M.; Iniesta, Á.; Cañada-García, J.E.; Valiente, O.; Aracil, B.; Arana, D.M.; Oteo-Iglesias, J. Emergence of NDM-1- and OXA-23-Co-Producing *Acinetobacter baumannii* ST1 Isolates from a Burn Unit in Spain. *Microorganisms* **2025**, *13*, 1149. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.