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Emergence of New Delhi Metallo-β-lactamase-9 (blaNDM-9) gene in Carbapenem-Resistant Acinetobacter baumannii Clinical Isolates in Africa

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Abstract

Background

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) are World Health Organization priority pathogens due to their significant role in hospital-acquired infections and multidrug resistance. The main carbapenem resistance mechanism in *A. baumannii* is the carbapenem-hydrolyzing oxacillinases (OXA-type carbapenemases); however, metallo-β-lactamases (MBLs) confer broader β-lactam drug resistance, threatening the effectiveness of available treatments.

Results

In this study, *A. baumannii* clinical isolates in Africa were recovered from patients in three Kenyan hospitals with skin and soft tissue, or throat infections. Bacterial identification and antimicrobial susceptibility testing were performed using the VITEK-2 system, and minimum inhibitory concentrations were interpreted according to CLSI guidelines. Whole-genome sequencing (Oxford Nanopore) and bioinformatics analysis revealed all isolates belonged to high-risk strain type ST2 and exhibited resistance to a range of antibiotics in multiple classes, including carbapenems. Three carbapenemase genes were identified: bla_{NDM-9}, bla_{OXA-23}, and bla_{OXA-66}, all chromosomally encoded, with bla_{NDM-9} embedded within a Tn125 transposon flanked by IS*Aba125*. Additionally, a biocide resistance gene, *qacEΔ1*, was identified on an integron, highlighting the role of mobile genetic elements in driving resistance to both antibiotics and antiseptics.

Conclusions

This study reports the first identification of New Delhi Metallo-β-lactamase-9 (NDM-9) producing *A. baumannii* clinical isolates in Africa. Enhanced surveillance and effective infection control strategies are urgently needed to mitigate the public health impact of highly resistant CRAB strains in Africa.

INTRODUCTION

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is a WHO priority pathogen due to resistance, limited treatment options and their association with hospital-acquired infections, which can have life-threatening outcomes [1]. These organisms are known for their ability to survive in hospital environments, biofilm formation capabilities and their genomic plasticity in acquiring resistance genes [2]. Treatment options for CRABs are severely limited, often requiring the use of older, more toxic agents like polymyxins (colistin) and tigecycline, which also face rising resistance rates [3]. CRABs have serious global implications due to the increased rates of morbidity, mortality and healthcare costs associated with these infections (4).

The predominant mechanism of carbapenem resistance in *A. baumannii* is the OXA-type carbapenemases. These are commonly classified as the class D β-lactamases. They include OXA-23, OXA-24/40 and OXA-58 β-lactamases and the naturally occurring OXA-51-like variants (which confer resistance when overexpressed, usually by an upstream insertion sequence like ISAba1) [5]. The class D β-lactamases are typically oxacillinases that weakly hydrolyze carbapenems, but their success in carbapenem resistance in diverse pathogens in the

Enterobacteriaceae family is based on the influence of insertion sequences upstream of the genes with plasmids mediating ease of transfer across isolates [6].

In comparison to OXA β-lactamases, metallo-β-lactamases (MBLs) confer higher and broader levels of resistance, evidenced by their ability to readily hydrolyze almost all β-lactams with the exception of aztreonam [7]. The dissemination of carbapenem genes is a global concern, especially where MBL-encoding genes are propagated among clinical strains. Currently, there are no β-lactamase inhibitors active against MBLs [8]. New Delhi Metallo-β-lactamases (NDMs) are among the most clinically relevant MBLs, which, due to their widespread propagation in plasmids as observed for variants such as NDM-1 [9] have received global attention.

NDM-1 is the most common NDM-type carbapenemase in CRAB isolates; however, strains producing other variants, including NDM-2, NDM-5, NDM-6, and more recently NDM-42, have been reported across the globe [10–14]. *bla*$_{NDM-9}$ differs by a single amino acid substitution (E152K) from the originating *bla*$_{NDM-1}$ [15]. The NDM-9 variant has been rarely detected in *A. baumannii*, though it has been observed in *K. pneumoniae* and *E. coli* [16, 17]. The earliest report of *bla*$_{NDM-9}$ is in a NDM-9-producing *Acinetobacter baumannii* isolate from France, where the gene was embedded within a Tn125 composite transposon carried on a 166-kb non-conjugative plasmid [18].

Amongst NDM-1, NDM-5 and NDM-9-producing *A. baumannii* strains, NDM-9 strains exhibited the highest minimum inhibitory concentrations (MICs) against the 5th generation cephalosporin, cefiderocol, demonstrating high-level activity of this carbapenemase toward this antibiotic, compared to other MBLs. [17]. Furthermore, Le Terrier et.al. 2023 report the *in-vitro* ineffectiveness of cefepime/taniborbactam against NDM-9-producing isolates. The presence of the NDM-9 variant among Enterobacteriaceae threatens the efficacy of these newer antibiotic and antibiotic/inhibitor therapies even though clinical trials have reported their efficacy against carbapenem-resistant isolates harboring other NDM variants [19].

This study presents both phenotypic and genomic characterization of four CRAB clinical isolates carrying the *bla*$_{NDM-9}$ gene, recovered between 2025-6 from patients recruited into an antimicrobial resistance surveillance study in Kenya. This paper presents the first report of NDM-9-producing *Acinetobacter baumannii* clinical isolates in Africa.

MATERIALS AND METHODS

Patients' clinical history

The four isolates were from patients hospitalized with either a throat infection or a skin and soft tissue infection. Table 1 describes the age, sex, clinical presentation, and infection type (either hospital acquired infection (HAI) or community acquired infection (CAI)).

Table 1
Clinical characteristics of the four isolates from three hospitals in Kenya

Sample ID	Region	Age	Sex	Clinical presentation	HAI/ CAI	Patient type	Year of sampling
KEN1943	Nairobi	25	M	Skin and soft tissue infection	HAI	In-patient	2025
KEN1954	Kisii	30	M	Skin and soft tissue infection	CAI	In-patient	2025
KEN2030	Lamu	64	M	Skin and soft tissue infection	CAI	In-patient	2026
KEN2074	Nairobi	8	F	Throat infection	HAI	In-patient	2026

Bacterial Isolation, Identification and Antimicrobial Susceptibility Testing

The *A. baumannii* isolates were recovered from the patients' clinical samples by standard microbiology processing. Bacterial identification was performed on the Vitek 2 system (bioMérieux, France) using the GN-ID card, while antimicrobial susceptibility testing was performed using the AST-GN83 card. The AST-GN83 panel screens for 11 antibiotics and antibiotic/inhibitor combinations, including: ampicillin/sulbactam, piperacillin/tazobactam, cefazolin, cefotaxime, ceftazidime, ceftriaxone, cefepime, meropenem, gentamicin, ciprofloxacin and trimethoprim/sulfamethoxazole. Interpretation of the minimum inhibitory concentrations (MICs) was based on the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2025) and the VITEK 2 Advanced Expert System.

DNA extraction and whole genome sequencing

Genomic DNA was extracted from isolates using the Quick-DNA™ Fungal/Bacterial Miniprep kit (Zymo Research). Libraries for whole genome sequencing were prepared using NEBNext reagents (New England Biolabs) and the Native Barcoding Kit (SQK-NBD114) (Oxford Nanopore Technologies). The final libraries were loaded onto an R10.4.1 flowcell, and sequencing was performed for 72 hrs on the MinION Mk1B device. Dorado v.0.9.0 (https://github.com/nanoporetech/dorado) super accuracy model, dna_r10.4.1_e8.2_400bps_sup @v5.0.0., was used for basecalling while filtering out low quality reads lower than Phred Quality score of 8.

Bioinformatics

The bioinformatics analysis involved several steps including *de novo* genome assembly using Autocycler v0.5.0 [20], an automated tool generating consensus long-read assemblies, utilizing multiple assemblers including Canu [21], Flye [22], Miniasm [23], Necat [24], Nextdenovo [25] and Raven [26], followed by a quality check with Quast v5.2.0 [27]. The assembled genomes were then subjected to Bakta v1.11.3 [28] for annotation and mlst v2.23.0 (Seeman, T) for Multi-locus sequence typing (MLST). Further characterization included screening for AMR genes using abritAMR v1.0.19 [29], identifying plasmid replicon types with a specialized *Acinetobacter baumannii* plasmid replicon typing scheme, AcinetobacterPlasmidTyping database v3.0, at a 95% nucleotide identity threshold [30], performing K and O locus typing for capsule antigens using Kaptive v3.1.0 [31] and screening for integrons using

Integron Finder [32]. Mob-typer v3.1.9 was used to identifier plasmid transferability [33]. Local BLASTn was used to identify insertion sequences based on query sequences. Multiple sequence alignment of the identified insertion sequences was performed using CLUSTAL W [34]. Core genome multilocus sequence typing (cgMLST) was carried out with chewBBACA v3.5.0 using a publicly available *Acinetobacter baumannii* core-genome MLST scheme, which includes 2,390 core genes within the species and was developed by Ridom.

RESULTS

Genomic features

Genome assembly yielded a complete circular chromosome for each isolate alongside a circularized plasmid. Chromosome and plasmid sizes as well as GC content are summarized in Table 2. All four isolates were assigned to *Acinetobacter baumannii* sequence type 2 (ST2) based on the Pasteur MLST scheme. Capsular typing identifies the K capsule type, KL234 and the O capsule type, OCLI across all isolates. The 11.1kb plasmids identified in all isolates were classified as R3-T4; replicons within the Rep3 family (T4 subtype). Mob-typing shows that the R3-T4 plasmid type is mobilizable, a plasmid that spreads by hijacking the transfer machinery of a conjugative plasmid resident in the host cell. However, no conjugative plasmid was identified in any of the isolates.

Table 2
Genomic features of the four NDM 9-producing *Acinetobacter baumannii* isolates

Isolate ID	No. of reads	Lenth of assembly	N50 / chromosome length	GC content	Plasmid length
KEN1943	149,222	4,090,296 bp	4,079,102	0.39	11,194 bp
KEN1954	707,856	4,039,764 bp	4,024,912	0.39	11,194 bp
KEN2030	302,775	4,053,115 bp	3,745,325	0.39	11,194 bp
KEN2074	166,525	4,031,529 bp	4,020,335	0.39	11,194 bp

Phenotypic antimicrobial susceptibility profiles

All four isolates were phenotypically resistant to all tested drugs: ampicillin/sulbactam, piperacillin/tazobactam, cefazolin, cefotaxime, ceftazidime, ceftriaxone, cefepime, meropenem, gentamicin, ciprofloxacin and trimethoprim/sulfamethoxazole (Table 3).

Table 3. Antimicrobial susceptibility profiles of the four NDM-9-producing *Acinetobacter baumannii* isolates. The symbol R denotes a resistance phenotype based on the CLSI interpretation

Drug class	Agents	KEN1943	KEN1954	KEN2030	KEN2074
Penicillin/combinations	Ampicillin/Sulbactam	>=32 R	>=32 R	>=32 R	>=32 R
	Piperacillin/Tazobactam	>=128 [R]	>=128 [R]	>=128 [R]	>=128 [R]
Cephalosporins	Cefazolin, Cefotaxime, Ceftazidime, Ceftriaxone, Cefepime	>=64 R	>=64 R	>=64 R	>=64 R
Carbapenems	Meropenem	>=16 R	>=16 R	>=16 R	>=16 R
Aminoglycosides	Gentamicin	>=16 R	>=16 R	>=16 R	>=16 R
Fluoroquinolone	Ciprofloxacin	>=4 R	>=4 R	>=4 R	>=4 R
Other	Trimethoprim/Sulfamethoxazole	>=320 R	>=320 R	>=320 R	>=320 R

Antimicrobial Resistance Genes (ARGs)

The following ARGs were identified on the chromosomes in all isolates: *bla*$_{NDM-9}$, *bla*$_{OXA-23}$, *bla*$_{OXA-66}$, *gyrA_S81L*, *cxpE*, *bla*$_{ADC-30}$, *abaF*, *ble*, *adeC*, *amvA*, *nreB*, *aph(3")-Ib*, *aph(6)-Id*, *sul2*, *parC_S84L*, *ant(3")-IIa*. *mph(E)*, *msr(E)*, *armA*, *sul1*, *qacEΔ1*, *aadA1*, *catB8* and *aac(6')-Ib'*. No ARGs were identified on the associated plasmids. Screening for integrons revealed several ARGs localized on a complete integron with 3 attC sites, recombination sites responsible for site-specific integration of gene cassettes, flanked by the *sul1* gene (Fig. 1).

Genetic environment of the bla$_{NDM-9}$ gene

The genetic environment around the *bla*$_{NDM-9}$ gene shows that the gene is embedded within a composite transposon flanked by IS*Aba125* insertion sequence on both ends. A three-base pair duplication region, GTG, was found surrounding both ends of the transposon, indicating the occurrence of a transposition event. The transposon inserted at the GTG target site within a LysM domain protein-encoding region, disrupting the gene by splitting it. This observation was consistent across all four isolates. However, in three of the study isolates, KEN1943, KEN1954 and KEN2074, the inserted composite transposon was 10,099 base pairs long while in one isolate, KEN2030, the composite transposon was 9,945 bp long, notably missing the co-chaperonin gene, *groES*. A schematic diagram showing the Tn125 structure in these isolates is in Fig. 2.

The two IS*Aba125* insertion sequences bracketing the ends of the composite transposon showed 100% nucleotide similarity between the Left Inverted Repeat (IRL) and Right Inverted Repeat (IRR) regions located at the termini and those present on the IS*Aba125* reference sequence (Accession AY751533). Single nucleotide polymorphisms (SNPs) were identified on the transposase gene present in both copies of the Is*Aba125* element at positions 313 (G -> A), 647 (G -> T) and 926 (A -> G) with one copy carrying extra SNPs at positions 833(A -> G), 836 (A->G), and 855 (T->C), in comparison to the IS*Aba125* reference sequence. Table 4 provides details of these variations with respect to the IS*Aba125* reference sequence.

Table 4
A summary table capturing the SNPs present on the 1087 bp IS*Aba125* insertion sequences flanking the composite transposon carrying the bla_{NDM-9}, as identified in the four isolates.

Position (X/1087)	Mutation	Number of copies with mutation	Copy 1 (furthest from NDM-9)	Copy 2 (adjacent to NDM-9)
313	G -> A	2	+	+
647	G -> T	2	+	+
833	A -> G	1	-	+
836	A -> G	1	-	+
855	T -> C	1	-	+
926	A -> G	2	+	+

Relatedness of the strains

A minimum spanning tree, generated based on cgMLST allelic differences, revealed that the isolates share < 20 allelic differences indicating potential epidemiological linkage. The three isolates containing the 10,099 bp Tn125 transposon differ by fewer than nine alleles, a threshold frequently employed to identify closely related isolates [35].

DISCUSSION

Globally, the bla_{NDM-9} carbapenemase gene has been identified in *E. coli*, *K. variicola*, *K. pneumoniae*, *Salmonella* species and *A. baumannii* isolates from Asia, Africa, and Europe [36–42]. The earliest reported *A. baumannii* with an NDM-9 gene was a clinical isolate from France 2013 carrying the gene on a 166kb plasmid [18]. To the best of our knowledge, this study is the first to report NDM-9-producing CRAB strains in Africa.

The Kenyan isolates were classified as strain type ST2, based on the Pasteur scheme, which belong to international clone 2 (IC2), a globally disseminated multidrug-resistant strain of *Acinetobacter baumannii*. ST2 is the most prevalent *A. baumannii* sequence type across the globe [43] and is known for its multidrug resistance and biofilm formation [44]. MDR ST2 *A. baumannii* strains identified in outbreak cases carrying OXA-23-like carbapenemases have been reported globally [45], highlighting the outbreak potential of this sequence type. Core genome MLST analysis shows that these study isolates are highly similar, indicating clonal expansion across the country. This finding is further emphasized by the identification of these clonal isolates in multiple sample types, including both throat and skin and soft tissue infections. Furthermore, their detection across different regions raises a concern of potential spread between healthcare facilities.

The genetic environment of the bla_{NDM-9} gene shows that it is located within a composite transposon, flanked by two copies of the insertion sequence, IS*Aba125*. Length variations were noted between the composite transposons, with 75% of the isolates exhibiting a 10,099 bp composite transposon, while one isolate presented a shorter 9,945 bp composite transposon. However, in all composite transposons, both copies of the IS*Aba125*, a 1087 bp element, were oriented in the same orientation, with identical inverted repeats and similar SNP sites. The composite transposon carrying the bla_{NDM-9} gene is the classical Tn125 transposon (10,099 bp), with the exception of the KEN2030 isolate which has lost the gene, *groES*, a co-chaperonin protein of *groEL* which have

protein folding functions and are therefore typically collocated on the classical transposon. Nonetheless, studies indicate that in several instances, *groEL* is capable of facilitating protein folding independently, without the involvement of the co-chaperonin *groES* [46]. Consistent with the Tn125 structure, there is a 93 bp spacer region between the NDM gene and the closest copy of the IS*Aba125*, together with a 3 bp direct repeat region bracketing the transposon on both ends [12]. This transposon has previously been documented in transposition events involving NDM-1 and NDM-2 genes as well as other β-lactamases in *A. baumannii* [47, 48]. Its presence in NDM-9 isolates denotes the structural adaptation of the Tn125 transposon for the mobilization of New Delhi metallo-β-lactamase genes.

This study identified conserved inverted repeat sequences at the termini of the Tn125 transposon, and substitution mutations within the transposase genes of the IS*Aba125* element. The findings are consistent with the previously described Tn125 structure [48]; however, prior studies did not specify mutations within the IS*Aba125*. This report identified the single nucleotide polymorphisms (SNPs) in the transposase genes within both copies of the IS*Aba125* elements. Functional annotation will be essential for evaluating the potential effects of these mutations on gene activity.

The 3-bp duplication region bracketing the Tn125 tranposon in the earlier 2013 French NDM-9 carrying *A. baumannii* isolate was a TTT present on a non-coding region within a plasmid [18]. In contrast, in all our study isolates, the Tn125 transposon was bracketed by a 3-bp duplication region (GTG). The transposon was inserted into a gene encoding a LysM domain-containing protein. Previous studies have shown that proteins containing lysin motifs (LysM) are extracellular and play a role in modifying or degrading the bacterial peptidoglycan layer [49]. The observation of a transposition event occurring at a coding region within the chromosome in the African isolates suggests that the dissemination of $bla_{\text{NDM-9}}$ in *A. baumannii* is related to Tn125's ability to identify transposition hotspots rather than a plasmid-based selectivity, as is common with most other NDM genes localized on plasmids [48].

Other than the carbapenem-resistant determinant, $bla_{\text{NDM-9}}$, these isolates possess a diverse array of antibiotic resistance genes against different classes of antibiotics, enhancing their multidrug resistance phenotype. These include antibiotic resistance genes (ARGs) and mutations conferring resistance to bleomycin(*ble*), carbapenems ($bla_{\text{NDM-9}}$, $bla_{\text{OXA-23,}}$ $bla_{\text{OXA-66}}$), quinolones (*gyrA_S81L, parC_S84L*), chloramphenicols (*catB8, cxpE*), cephalosporins ($bla_{\text{ADC-30}}$), fosfomycins (*abaF*), azithromycins/erythromycins (*mphE, msrE*), sulfonamides (*sul1,sul2*) and aminoglycosides (*armA, aadA1, aph(3")-Ib, aph(6)-Id", aac(6')-Ib', ant(3")-IIa*). A gene belonging to the RND superfamily of efflux pumps (*adeC*) which contribute to multidrug resistance, biofilm formation and virulence was also detected [50]. Another efflux pump, *amvA*, which has been shown to induce tolerance to disinfectants in clinical *A. baumannii* isolates and influence biofilm formation was also detected [51, 52]. Multiple resistance determinants that confer resistance against single antibiotic classes were observed most notably for aminoglycosides, chloramphenicols, macrolides and carbapenems.

There was co-carriage of three carbapenem-resistance determinants, one MBL and two OXA-type carbapenemases ($bla_{\text{NDM-9}}$, $bla_{\text{OXA-23,}}$ $bla_{\text{OXA-66}}$). A previous study conducted in Kenya between 2015–2018 found several CRAB non-ST2 strains with three determinants, two OXA variants and NDM-1, though only carbapenem-resistant ST2 isolates carrying OXA carbapenemase genes ($bla_{\text{OXA-23,}}$ $bla_{\text{OXA-66}}$) were identified [53]. The detection of $bla_{\text{NDM-9}}$ gene in isolates collected between 2025–2026 indicates the emergence of NDM-9 in the region. The addition of NDMs in CRAB strains with OXA carbapenemases potentially elevates the hydrolytic capabilities of

these strains allowing for more effective hydrolysis of aztreonam and fifth generation cephalosporins [17]. More specifically, NDM-9 has been proven to render the promising MBL inhibitor, taniborbactam, ineffective [19]. This accumulation of resistance determinants within CRAB isolates underscores the severity of multi-drug resistance threat posed by these strains. The consequence of having multiple resistance genes targeting single drug classes is a CRAB with high-level multidrug-resistance making them difficult-to treat and increasing disease morbidity and mortality [54].

The isolates possessed a class 1 integron carrying up to four antimicrobial resistance genes, ARGs (*sul1, aadA1, catB8 and aac(6')-Ib'*). In addition to these ARGs, the integron carried the *qacEΔ1* gene, a gene known to confer resistance to various antiseptics and disinfectants. A One Health study examining *A. baumannii-calcoaceticus* isolates showed that the *qacEΔ1* gene was exclusively identified in clinical isolates suggesting a potential link to extensive biocide usage in healthcare settings [55]. The class 1 integron is a mobile genetic element known for its role in the dissemination of antimicrobial resistance [56]. The acquisition of this integron carrying *qacEΔ1* provides these isolates with both antimicrobial and antiseptic resistance determinants that enhance the survival and adaptability of these MDR strains in clinical settings. This phenomenon highlights the evolutionary strategies these strains employ to evade not only antibiotics but also biocidal agents, giving them the ability to persist in regularly decontaminated hospital environments and further complicating infection control efforts.

Of the *A. baumannii* plasmid types, the R3-type plasmids, are the most diverse carrying resistance determinants. Some R3-type plasmids have been found to harbor carbapenem resistance genes including $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, $bla_{\text{OXA-58}}$, and bla_{NDM} [57]. However, the identified R3-type plasmids in our study did not have any AMR genes but had several ORFs including a septicolysin gene coding for a pore-forming toxin [58] and several toxins (BrnT family toxin, Addiction module toxin RelE) and a toxin-antitoxin system. We hypothesize that the key role of this R3-type plasmid is to ensure continued selection for plasmid-containing cells, by initiating a killing mechanism for any daughter cell(s) that loses the plasmid. This plasmid type is characterized as being mobilizable, suggesting potential plasmid transferability among strains [59]. It is crucial to continue monitoring this plasmid type in these multi-drug-resistant strains for any evolution in acquiring AMR genes through mobile elements encoding ARGs on the host chromosome.

CONCLUSION

This study reports the first detection of $bla_{\text{NDM-9}}$ producing *Acinetobacter baumannii* clinical isolates in Africa, belonging to the globally prevalent MDR ST2 clone. The $bla_{\text{NDM-9}}$ gene was found embedded within a Tn125 transposon on the chromosome highlighting the role of mobile genetic elements in carbapenem resistance. The detection of the *qacEΔ1* gene implicated in antiseptic resistance, also on a mobile element, highlights the adaptability of these strains within hospital environments. The emergence of NDM-9 producing *A. baumannii* strains in Africa heightens the risk of extensive resistance to available last-line carbapenem/combination therapies, undermining the efficacy of newer antibiotics like cefiderocol and cefepime/taniborbactam, thereby limiting available therapeutic options. The findings from this study underscore the need for enhanced surveillance to monitor and track NDM-9 variants and their resistance profiles, including the localization and dissemination of these ARGs. Such efforts will provide essential data on the risk of carbapenem resistance transmission and inform ongoing assessments of therapeutic efficacy.

Declarations

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Ethical approval

The study was conducted in accordance with the Declaration of Helsinki (2013) and approved by the Kenya Medical Research Institute (KEMRI) Scientific and Ethics Review Unit (#2767), the Walter Reed Army Institute of Research (WRAIR) Institutional Review Board (#2089), and the U.S. Army Medical Research and Materiel Command, Office of Research Protection, Human Research Protections Office (USAMRMC ORP HRPO) (Log#A-18129). Written informed consent was obtained from the participating patients prior to sample collection and data analysis.

Consent for publication

All authors have reviewed and approved the final version of the manuscript and consented to its publication.

Data Availability

The complete genome assemblies of the *Acinetobacter baumannii* isolates have been deposited in the NCBI GenBank database under BioProject ID: PRJNA1451447.

Competing interests

The authors declare no competing interests.

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Figures

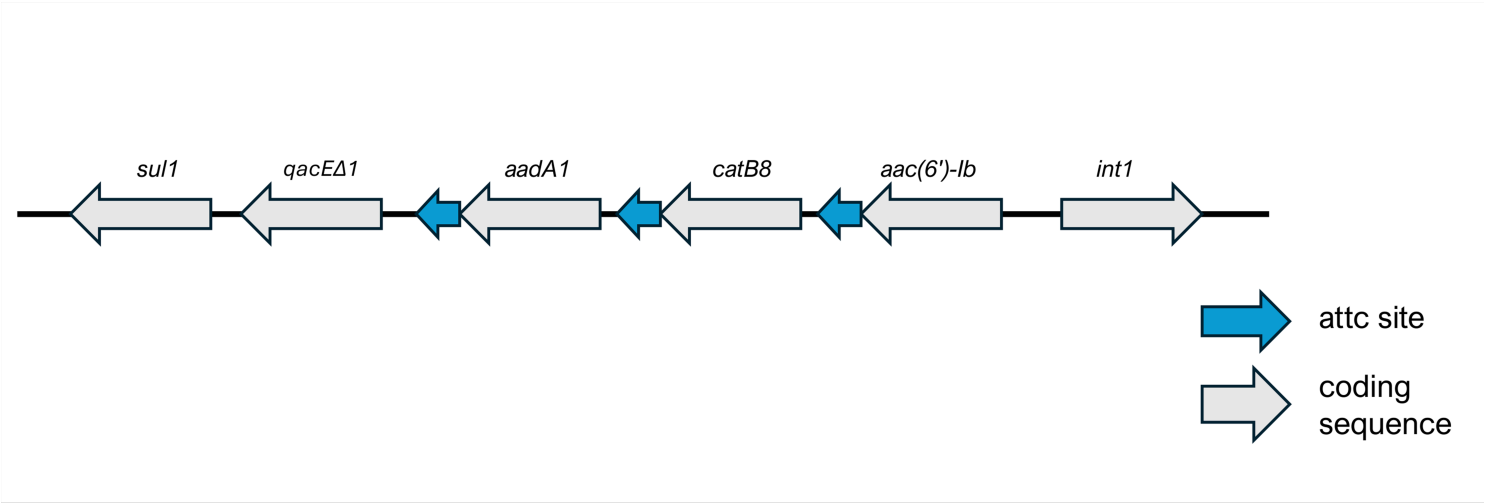


Figure 1

Linear map of the integron locus present in all four *A. baumannii* isolates showing the integrase gene (*int1*), the three attC sites (marked in blue) and the ARGs present: *catB8* (resistance to chloramphenicols), *aadA1* & *aac(6')-Ib* (resistance to aminoglycosides), qacE*Δ1* (resistance to antiseptics) and *sul1* (resistance to sulfonamides).

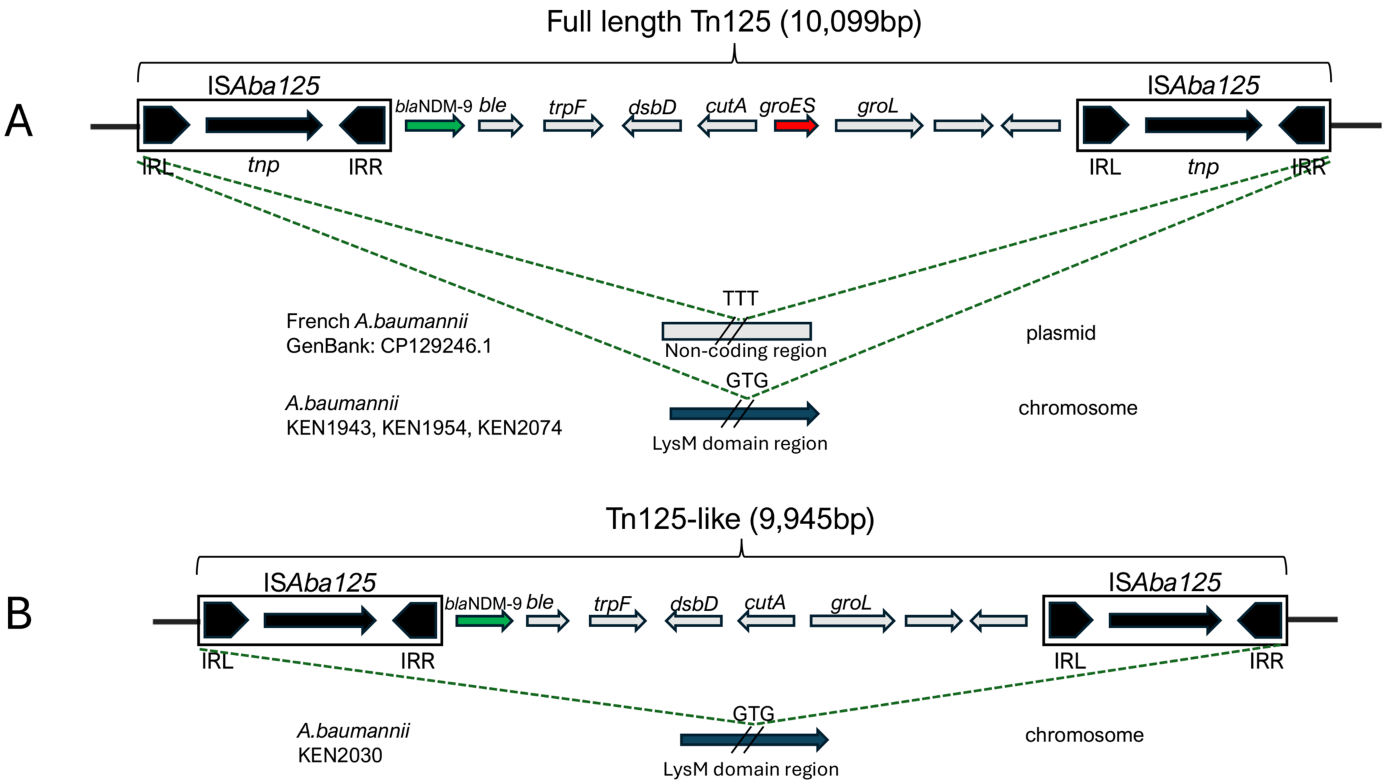


Figure 2

A) Linear schematic of the classical Tn125 transposon (size 10,099 bp) carrying the NDM-9 gene (highlighted in green). The IS*Aba125* region, composed of Left Inverted Repeat region (IRL), transposase gene (tnp) and Right Inverted Repeat region (IRR) are shown (bordered by a box) flanking both ends of the transposon. The open reading frames are represented by arrows in gray with the lengths drawn to scale. Annotation of the genome identified several genes on the transposon including transposase enzyme (*tnp***)**, chaperonin proteins (*groL* & *groES*), divalent-cation tolerance homolog (*cutA*), reductase (*dsbD*), phosphoribosyl anthranilate isomerase (*trpF*) and the ARGs: *ble* and bla_{NDM-9}. Characterization of Tn125 transposition events is shown for NDM-9 producing *A. baumannii* isolates CP129246.1 and three study isolates: KEN1943, KEN1954 and KEN2074. The 3 bp target sites are written in uppercase above the arrow/rectangle box, and the target site name on the bottom side. B) Linear representation of the shorter Tn125-like transposon (9945 bp), identified in one study isolate, KEN2030. This transposon region closely resembles the full-length sequence but lacks the co-chaperonin gene *(gro*ES) highlighted in red in Figure 2A.

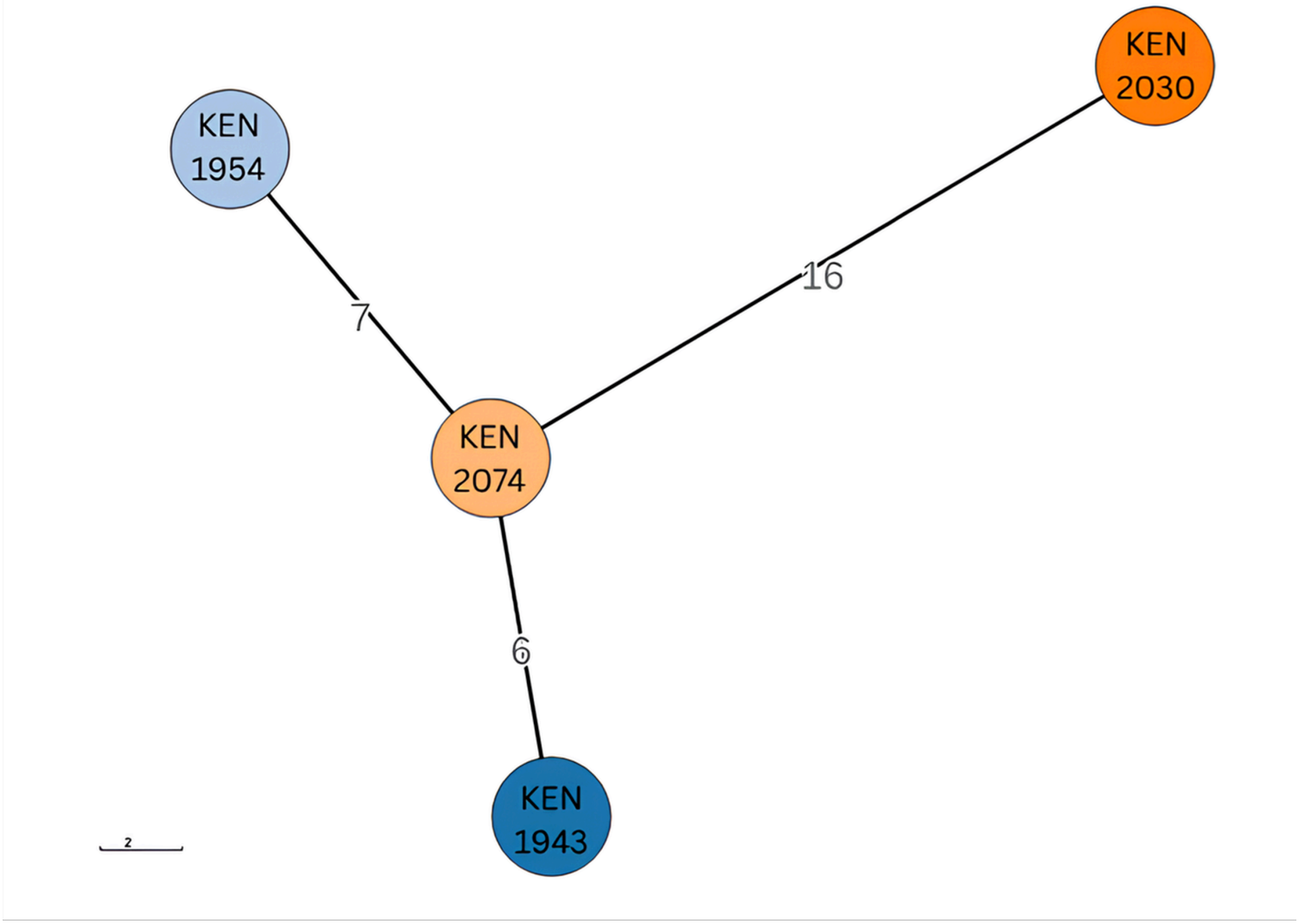


Figure 3

Minimum spanning tree (drawn to scale) based on cgMLST allelic differences of the four study isolates. The branch numbers indicate allelic differences observed.