European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: 3105-0409
Website: https://farmclin.com/

Original Research Article

Genomic Plasticity and Carbapenemase Mobility in Extensively Drug-Resistant Acinetobacter baumannii SO_1077_3 Revealed by Long-Read Sequencing

Article History:

Name of Author:
Janakiram Bobbillapati[1], Monika Ranjan Tatapudi[2], J. Naveena Lavanya Latha*[3] AS Smiline Girija[1]*

Affiliation: *[1]Department of Microbiology, Saveetha dental college, Saveetha university, Chennai.*
[2]Department of Child health nursing, KSR college of nursing, Guntur, A.P.
*[*3]Department of Biotechnology, Krishna University, Machilipatnam, Andhra Pradesh*

Corresponding Author:
Dr. J. Naveena Lavanya Latha
Dr. AS Smiline Girija

Received: 13-10-2025
Revised: 24-10-2025
Accepted: 11-11-2025
Published: 26-11-2025

Abstract: The increasing emergence of extensively drug-resistant (XDR) Acinetobacter baumannii poses a major global health threat, largely driven by mobile genetic elements and carbapenemase dissemination. In this study, long-read whole genome sequencing was performed using Oxford Nanopore technology to generate a complete genome assembly of a clinically isolated XDR A. baumannii SO_1077_3 strain. Whole genome assembly resulted in a closed circular chromosome and resolved plasmid structures with high coverage depth. Structural genome analysis identified multiple insertion sequences, resistance islands, prophage regions, and plasmid-borne carbapenemase genes including OXA-24 and OXA-58. Genomic context analysis revealed insertion sequence–mediated mobilization of β-lactamase genes, highlighting mechanisms underlying carbapenem resistance dissemination. The complete genome architecture provides critical insights into structural determinants of antimicrobial resistance and horizontal gene transfer. This study demonstrates the utility of long-read sequencing for resolving complex resistance loci and mobile elements in high-risk XDR pathogens.

Keywords: Long-read sequencing; Oxford Nanopore; Complete genome assembly; Carbapenemase; XDR Acinetobacter baumannii, Anti microbial resistance (AMR).

INTRODUCTION

The rapid emergence and global dissemination of extensively drug-resistant (XDR) *Acinetobacter baumannii* has become one of the most alarming developments in contemporary clinical microbiology. Recognized by the World Health Organization (WHO) as a "critical priority" pathogen for research and development of new antibiotics, carbapenem-resistant *A. baumannii* represents a formidable threat in healthcare settings worldwide. This opportunistic Gram-negative coccobacillus is particularly problematic in intensive care units (ICUs), where it causes ventilator-associated pneumonia, bloodstream infections, wound infections, and urinary tract infections, especially among immunocompromised and critically ill patients. The organism's remarkable ability to survive under desiccation, persist on abiotic surfaces, and form biofilms contributes significantly to nosocomial transmission. Over the past two decades, outbreaks of multidrug-resistant (MDR),

extensively drug-resistant (XDR), and even pandrug-resistant (PDR) strains have been reported across Asia, Europe, the Americas, and the Middle East, with increasing frequency in low- and middle-income countries. Mortality rates associated with severe infections caused by carbapenem-resistant *A. baumannii* often exceed 40–60%, underscoring the urgent need for comprehensive genomic and molecular investigations (Peleg et al., 2008; Antunes et al., 2014; Tacconelli et al., 2018).

The success of XDR *A. baumannii* as a nosocomial pathogen is largely attributable to its extraordinary genomic plasticity and its capacity to acquire, accumulate, and express a broad spectrum of antimicrobial resistance (AMR) determinants. Resistance in *A. baumannii* is mediated through multiple mechanisms, including enzymatic inactivation of antibiotics (e.g., OXA-type carbapenemases), overexpression of efflux pumps such as AdeABC, target site mutations in gyrA and parC, permeability defects due to porin alterations, and lipopolysaccharide (LPS) modifications conferring colistin resistance. The carbapenem-hydrolyzing class D β-lactamases (CHDLs), including OXA-23, OXA-24/40, OXA-51-like, and OXA-58, play a central role in carbapenem resistance and are frequently associated with insertion sequences that enhance gene expression (Poirel & Nordmann, 2006; Evans & Amyes, 2014). Furthermore, aminoglycoside resistance genes such as armA and rmtB, as well as mutations affecting the Lpx pathway, have contributed to the dwindling efficacy of last-resort antibiotics, including colistin. The cumulative effect of these mechanisms results in strains resistant to nearly all available antimicrobial classes, severely limiting therapeutic options.

A defining feature of *A. baumannii* is its ability to mobilize resistance genes via horizontal gene transfer. Mobile genetic elements (MGEs), including plasmids, transposons, insertion sequences (IS), integrons, and genomic resistance islands (such as AbaR-type islands), facilitate rapid adaptation under antibiotic pressure. Insertion sequences like ISAba1 and ISAba3 are known to upregulate adjacent β-lactamase genes, increasing carbapenem resistance levels. Plasmids often harbor clusters of AMR genes and may be conjugative, enabling inter-strain dissemination. Additionally, prophages and genomic islands contribute to genome diversification and may carry virulence-associated determinants. The interplay between chromosomal mutations and horizontally acquired elements creates a dynamic and evolving resistance architecture, making genomic surveillance essential for understanding outbreak epidemiology and resistance evolution (Fournier et al., 2006; Hamidian & Hall, 2018).

Whole genome sequencing (WGS) has revolutionized the study of antimicrobial resistance and pathogen evolution. Short-read sequencing platforms, particularly Illumina-based technologies, have been widely adopted due to their high accuracy, throughput, and relatively low cost. These platforms have significantly advanced our understanding of the resistome and virulome of *A. baumannii*, enabling rapid identification of resistance genes, multilocus sequence typing (MLST), and phylogenomic analysis. Numerous epidemiological investigations have relied on short-read WGS to trace hospital outbreaks, identify international clones (IC I, IC II, IC III), and characterize high-risk lineages. However, despite their strengths, short-read assemblies often produce fragmented draft genomes consisting of dozens to hundreds of contigs, particularly in organisms rich in repetitive sequences and mobile elements (Didelot et al., 2012).

The limitations of short-read assemblies become especially evident when analyzing complex genomic structures such as resistance islands, plasmids, and insertion sequence–flanked regions. Repetitive elements longer than the read length cannot be accurately resolved, leading to assembly gaps or misassemblies. Consequently, it becomes challenging to determine whether specific AMR genes are located on the chromosome or plasmids, whether they are associated with transposons, or whether multiple copies exist within the genome. For example, OXA-type carbapenemase genes are frequently embedded within transposable elements bracketed by identical insertion sequences, which cannot be reliably assembled using short reads alone. This structural ambiguity limits our ability to interpret gene mobility, assess transmission potential, and understand the genomic context driving high-level resistance. Moreover, plasmid reconstruction from short-read data remains problematic, as contigs derived from plasmids may be incorrectly merged with chromosomal sequences or remain unlinked (Arredondo-Alonso et al., 2017).

Long-read sequencing technologies, such as those developed by Oxford Nanopore Technologies and Pacific Biosciences, have emerged as transformative tools for resolving these challenges. Long-read platforms generate reads that span thousands to hundreds of thousands of base pairs, enabling the assembly of complete, circularized bacterial genomes and plasmids. These technologies allow direct resolution of repetitive regions, insertion sequences, structural rearrangements, and complex genomic islands. In *A. baumannii*, long-read sequencing has facilitated the accurate mapping of AbaR resistance islands, identification of plasmid-borne carbapenemase genes, and characterization of large chromosomal inversions and recombination events. The ability to generate closed genomes provides an

unparalleled view of the structural architecture underlying antimicrobial resistance and virulence (Wick et al., 2017).

Structural genomics—the study of genome organization, architecture, and large-scale genomic features—has become increasingly important in understanding the evolution and dissemination of resistance in XDR pathogens. Unlike functional genomics, which focuses primarily on gene presence and expression, structural genomics examines how genes are arranged, mobilized, and regulated within the genome. In the context of *A. baumannii*, this includes identifying insertion sequence–mediated promoter activation, transposon integration sites, plasmid replicon types, prophage insertions, and genomic rearrangements. These structural elements often determine whether a resistance gene is stable within the chromosome or capable of horizontal transfer. Therefore, resolving the complete genomic landscape is critical for understanding both local outbreak dynamics and global dissemination patterns.

In addition to antimicrobial resistance, structural genomic features influence virulence and environmental persistence. Biofilm-associated genes, outer membrane proteins, iron acquisition systems, and stress response regulators may be embedded within genomic islands or plasmids. Structural rearrangements can affect gene regulation, fitness, and adaptability under antibiotic pressure. Thus, a complete genome assembly not only clarifies resistance architecture but also provides insight into pathogenic potential and survival strategies. The integration of long-read sequencing data into clinical microbiology therefore represents a significant step toward precision epidemiology and improved infection control strategies.

Given the escalating threat posed by XDR *A. baumannii* and the inherent limitations of short-read draft assemblies, there is a compelling need to generate complete, high-quality genome assemblies that resolve the structural context of resistance determinants. The present study aims to employ long-read whole genome sequencing to produce a fully closed genome of an XDR *A. baumannii* clinical isolate. By resolving chromosomal and plasmid structures, identifying resistance islands and mobile genetic elements, and mapping the genomic context of key carbapenemase genes, this work seeks to elucidate the structural architecture underlying extensive drug resistance. Such comprehensive genomic resolution will enhance our understanding of resistance evolution, inform surveillance efforts, and contribute to the development of targeted strategies to combat this critical priority pathogen.

2. Materials and Methods

2.1 Bacterial Isolation and Antimicrobial Susceptibility Testing

A clinical isolate of *Acinetobacter baumannii* (designated SO_10770_3) was recovered from a hospitalized patient using standard microbiological procedures. Species identification was initially performed using conventional biochemical assays and subsequently confirmed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS).

Antimicrobial susceptibility testing (AST) was performed using the broth microdilution method according to guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2023). Minimum inhibitory concentrations (MICs) were interpreted using CLSI breakpoints. Based on susceptibility profiles across multiple antimicrobial classes, the isolate was classified as extensively drug-resistant (XDR) following internationally standardized criteria (Magiorakos et al., 2012).

2.2 High-Molecular-Weight DNA Extraction

Genomic DNA suitable for long-read sequencing was extracted using a phenol–chloroform protocol optimized for high-molecular-weight DNA recovery. Care was taken to minimize mechanical shearing during extraction.

DNA purity was evaluated using NanoDrop spectrophotometry (A260/A280 ratio), and concentration was quantified using Qubit fluorometric assays. Integrity of genomic DNA was confirmed by agarose gel electrophoresis, ensuring fragment sizes predominantly exceeded 20 kb to facilitate optimal long-read sequencing performance.

2.3 Library Preparation and Nanopore Sequencing

Sequencing libraries were prepared using the ligation sequencing kit (Oxford Nanopore Technologies) according to the manufacturer's protocol. Libraries were loaded onto an R9.4.1 flow cell and sequenced using the MinION platform developed by Oxford Nanopore Technologies. Real-time sequencing was conducted using MinKNOW software.

Long-read sequencing was selected to enable resolution of repetitive insertion sequences, transposons, and plasmid structures that are frequently collapsed in short-read assemblies (Wick et al., 2017). Sequencing generated approximately 0.83 GB of raw long-read data.

2.4 Basecalling and Quality Control

Raw FAST5 files were basecalled using Guppy basecaller in high-accuracy mode. Adapter trimming was performed using Porechop (v0.2.3), and reads were filtered using NanoFilt (v2.8.0), retaining reads with a minimum quality score of $Q \geq 10$.

Read quality metrics, including length distribution and quality score profiles, were evaluated to ensure suitability for de novo assembly. Quality filtering was performed to minimize downstream assembly errors while preserving long-read continuity.

2.5 De Novo Genome Assembly and Assembly Assessment

Filtered reads were assembled de novo using Flye assembler (version X.X), optimized for long, error-prone reads and repetitive bacterial genomes (Kolmogorov et al., 2019).

Assembly statistics including total genome size, contig number, and structural completeness were evaluated using QUAST. The final assembly resulted in three contigs corresponding to a large chromosomal sequence (~4 Mb) and two plasmid sequences. Circularization status was assessed based on assembly graph analysis.

2.6 Genome Polishing

To improve consensus accuracy, the draft assembly was polished iteratively. Initial polishing was performed using Racon for long-read consensus correction (Vaser et al., 2017), followed by neural network–based polishing using Medaka. Multiple rounds of polishing were applied to reduce insertion–deletion errors and improve base-level accuracy.

2.7 Genome Annotation and Functional Characterization

Structural annotation was conducted using Prokka for rapid prokaryotic genome annotation (Seemann, 2014). Functional annotation included identification of coding sequences (CDS), transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), and regulatory features.

Gene ontology classification and protein functional assignments were derived from DIAMOND BLASTP searches against curated bacterial databases. Species identity and genomic relatedness were further validated using Average Nucleotide Identity (ANI) analysis and digital DNA–DNA hybridization (dDDH) via the Type (Strain) Genome Server (TYGS).

2.8 Detection of Antimicrobial Resistance Genes

Antimicrobial resistance (AMR) genes were identified using the Comprehensive Antibiotic Resistance Database (CARD) and its Resistance Gene Identifier (RGI) tool (Alcock et al., 2020), applying default curated thresholds. A total of 395 AMR-associated hits were identified.

To assess mobility potential, 500 bp upstream and downstream flanking regions of identified AMR genes were extracted and screened against the ACLAME database to detect mobile genetic element (MGE) signatures.

2.9 Identification of Mobile Genetic Elements and Structural Features

Insertion Sequences (IS Elements)

Insertion sequences were identified using the ISfinder database (Siguier et al., 2006), enabling classification of IS families associated with carbapenemase genes.

Genomic Islands

Genomic islands were predicted using IslandViewer 4 (Bertelli et al., 2017), integrating sequence composition and comparative genomics approaches to detect horizontally acquired regions.

Prophage Regions

Prophage sequences were identified using PHASTER, which detects intact and incomplete bacteriophage regions within bacterial genomes (Arndt et al., 2016).

Plasmid Identification and Replicon Typing

Plasmid sequences were identified from assembly contigs based on size, coverage, and homology. Replicon typing and mobility classification were performed using Mob-suite (Robertson & Nash, 2018), allowing prediction of plasmid transfer potential.

2.10 Comparative Genomics and Phylogenetic Analysis

Comparative genomic visualization was conducted using BLAST Ring Image Generator (BRIG) to assess genomic similarity against reference strains.

Species confirmation and phylogenetic positioning were determined using:

- Average Nucleotide Identity (ANI) analysis
- Digital DNA–DNA hybridization (dDDH) via TYGS

RESULTS

3.1 Nanopore Long-Read Sequencing Output and Quality Control

Nanopore sequencing of *Acinetobacter baumannii* strain SO_10770_3 generated approximately 0.83 GB of long-read data. Raw reads were filtered using NanoFilt (minimum quality score ≥10), and adapters were removed using Porechop (v0.2.3). Post-filtering statistics are summarized in Table 1.

The filtered dataset retained high-quality long reads suitable for de novo assembly, with sufficient read length distribution to span repetitive insertion sequence regions and structural rearrangements. The generated long-read dataset (Table 1) provided sufficient depth and read length to resolve repetitive genomic regions and structural mobile elements commonly associated with antimicrobial resistance in *A. baumannii*.

Table 1. Nanopore Read Statistics

Strain	SO_10770_3
Total data generated	0.83 GB
Minimum quality threshold	Q ≥10
Adapter trimming tool	Porechop v0.2.3
Quality filtering tool	NanoFilt v2.8.0

3.2 Long-Read Genome Assembly and Structural Validation

De novo assembly using long-read data resulted in a draft genome of approximately ~4 Mb. The assembly produced three contigs, comprising:

- One large chromosomal contig (~3.8–3.9 Mb)
- Two smaller contigs identified as plasmids

BLAST-based validation of the longest contig demonstrated strong homology with *Acinetobacter baumannii* ATCC19606. The two smaller contigs were confirmed as plasmid sequences based on BLAST alignment patterns (Figures 1 and 2).

Figure 1: Assembly graph visualization (Bandage plot showing chromosome and plasmid separation)

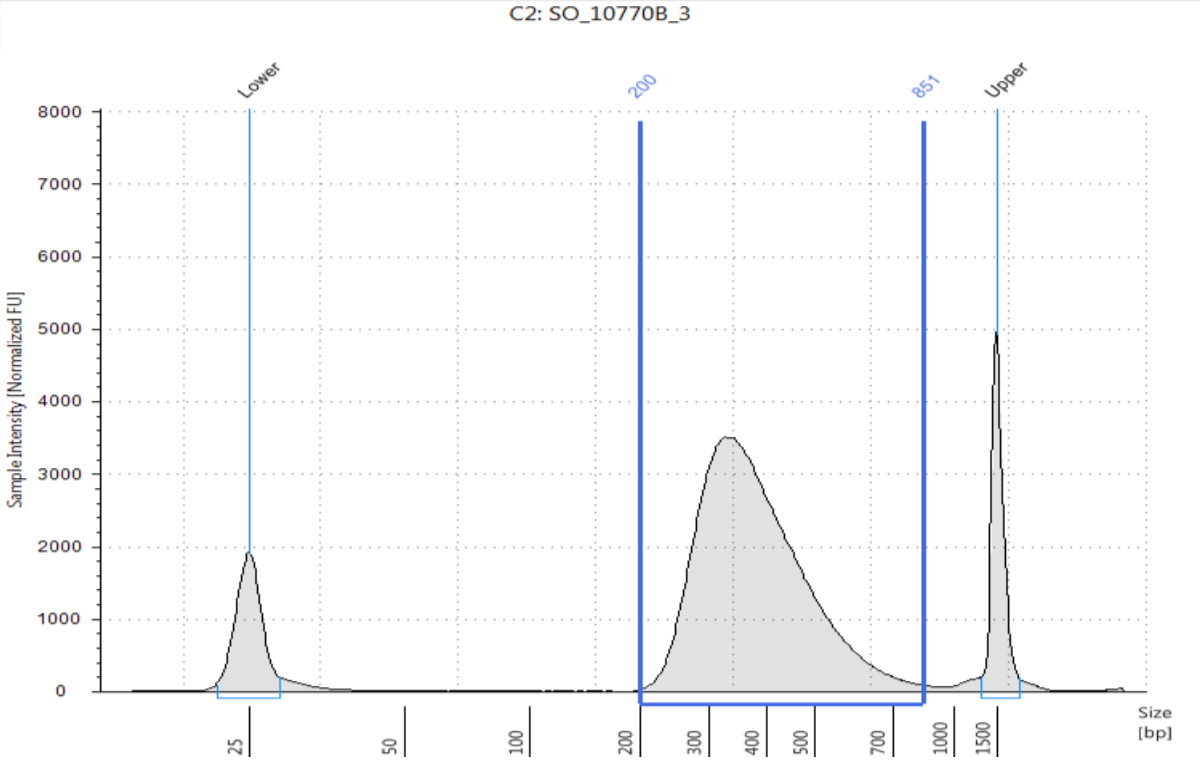


Figure 2: BLAST homology plot of longest contigs

Figure 2a NCBI NR blast result for contig1 (SO_10770_3)

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Acinetobacter baumannii DNA, complete genome, strain: IOMTU 433	Acinetobacter ...	2.406e+05	3.787e+05	100%	0.0	99.76%	4000970	AP014649.1
Acinetobacter baumannii strain VB16141 chromosome, complete genome	Acinetobacter ...	2.396e+05	3.774e+05	100%	0.0	99.63%	4082961	CP040050.1
Acinetobacter baumannii strain VB35575 chromosome, complete genome	Acinetobacter ...	2.385e+05	3.788e+05	100%	0.0	99.75%	4031418	CP040087.1
Acinetobacter baumannii ACICU, complete genome	Acinetobacter ...	2.107e+05	3.652e+05	97%	0.0	98.00%	3904116	CP000863.1
Acinetobacter baumannii strain KSK18 chromosome, complete genome	Acinetobacter ...	2.082e+05	3.787e+05	99%	0.0	99.72%	4095769	CP072290.1
Acinetobacter baumannii strain KSK20 chromosome, complete genome	Acinetobacter ...	2.082e+05	3.786e+05	99%	0.0	99.72%	4095769	CP072300.1
Acinetobacter baumannii strain KSK10 chromosome, complete genome	Acinetobacter ...	2.082e+05	3.786e+05	99%	0.0	99.72%	4096957	CP072280.1

Figure 2b: NCBI NR blast result for contig2 (SO_10770_3)

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Escherichia coli strain 6409 plasmid p6409-202.186kb, complete sequence	Escherichia coli	16643	49691	100%	0.0	100.00%	193908	CP010373.2
Acinetobacter indicus strain C15_T chromosome, complete genome	Acinetobacter in...	16637	72385	100%	0.0	99.99%	3242790	CP048654.1
Acinetobacter baumannii strain ABF9692 plasmid pABF9692, complete sequence	Acinetobacter b...	15989	20829	100%	0.0	99.97%	264805	CP048828.1
Acinetobacter baumannii DNA, complete genome, strain: IOMTU 433	Acinetobacter b...	15793	18636	100%	0.0	100.00%	4000970	AP014649.1
Acinetobacter baumannii strain KSK20 chromosome, complete genome	Acinetobacter b...	15793	18653	100%	0.0	100.00%	4095769	CP072300.1
Acinetobacter baumannii strain KSK18 chromosome, complete genome	Acinetobacter b...	15793	18653	100%	0.0	100.00%	4095769	CP072290.1

Figure 2c: NCBI NR blast result for contig3 (SO_10770_3)

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Acinetobacter baumannii strain CI415 chromosome, complete genome	Acinetobacter b...	3664	4364	100%	0.0	99.95%	3965259	CP071763.1
Klebsiella pneumoniae strain E16KP0301 plasmid pE16KP0301-9, complete sequence	Klebsiella pneu...	3380	4377	100%	0.0	100.00%	2278	CP052256.1
Acinetobacter baumannii strain ABAY15001 plasmid pABAY15001_6E, complete sequence	Acinetobacter b...	3057	4377	100%	0.0	100.00%	2278	MK386684.1
Acinetobacter baumannii strain Res13-Abat-PEA21-P4-01-A plasmid unnamednovel_2, complete sequ...	Acinetobacter b...	2905	4474	100%	0.0	99.50%	5242	CP062923.1
Acinetobacter baumannii strain E-072658 plasmid p2E072658, complete sequence	Acinetobacter b...	2307	2476	56%	0.0	99.29%	6018	CP061712.1
Acinetobacter wuhouensis strain WCHAW010062 plasmid p2_010062, complete sequence	Acinetobacter ...	2302	3978	56%	0.0	99.22%	28438	CP033122.1

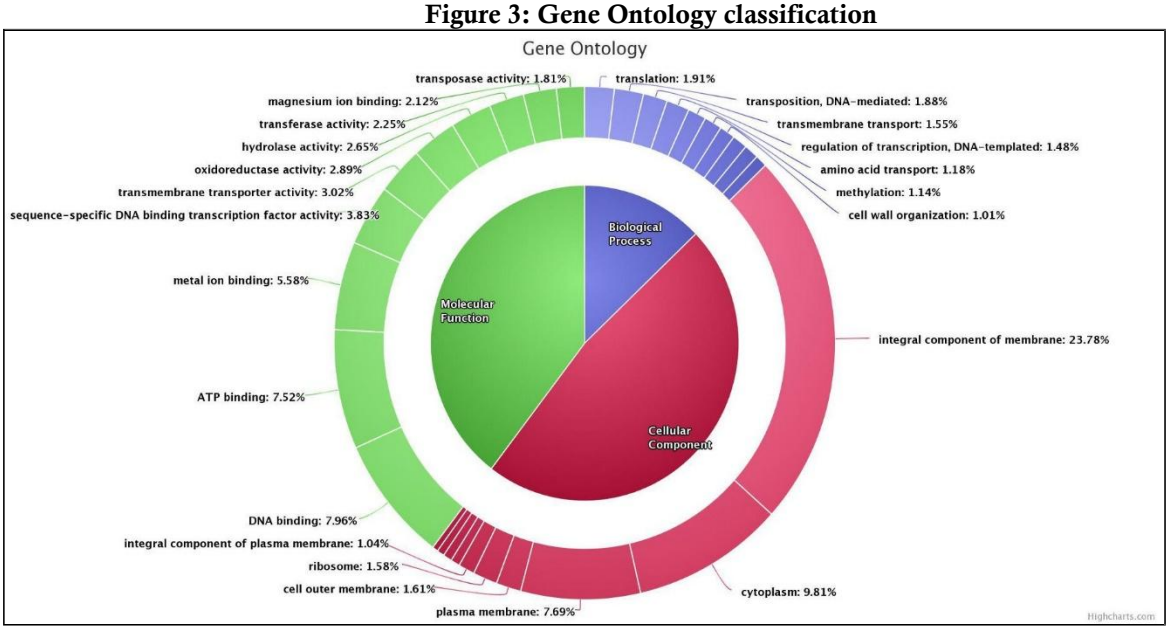
Nanopore long reads enabled structural resolution of chromosomal and plasmid sequences without fragmentation, confirming the presence of extrachromosomal elements and resolving repetitive regions that are typically collapsed in short-read assemblies.

3.3 Structural Genome Annotation

Gene prediction using Prokka identified 3,844 predicted proteins, of which 3,842 were successfully annotated using DIAMOND BLASTP against the UniProt Bacterial database (≥30% identity threshold).

Long-read assembly facilitated accurate mapping of gene clusters and repetitive elements without contig breaks, improving structural annotation fidelity.

Figure 3: Gene Ontology classification



3.4 Taxonomic Validation and Genome Similarity

DNA–DNA Hybridization (TYGS)

Genome-based phylogenetic analysis using the TYGS server showed that strain SO_10770_3 clustered closely with

Acinetobacter baumannii ATCC19606, with a d4 value of 81.5% (Figure-4).

Average Nucleotide Identity (ANI)

ANI analysis against *A. baumannii* ATCC19606 revealed a mean identity of 97.91%, confirming species-level classification (Figure 5).

Figure 4: Whole genome level phylogenetic tree for sample SO_10770_3

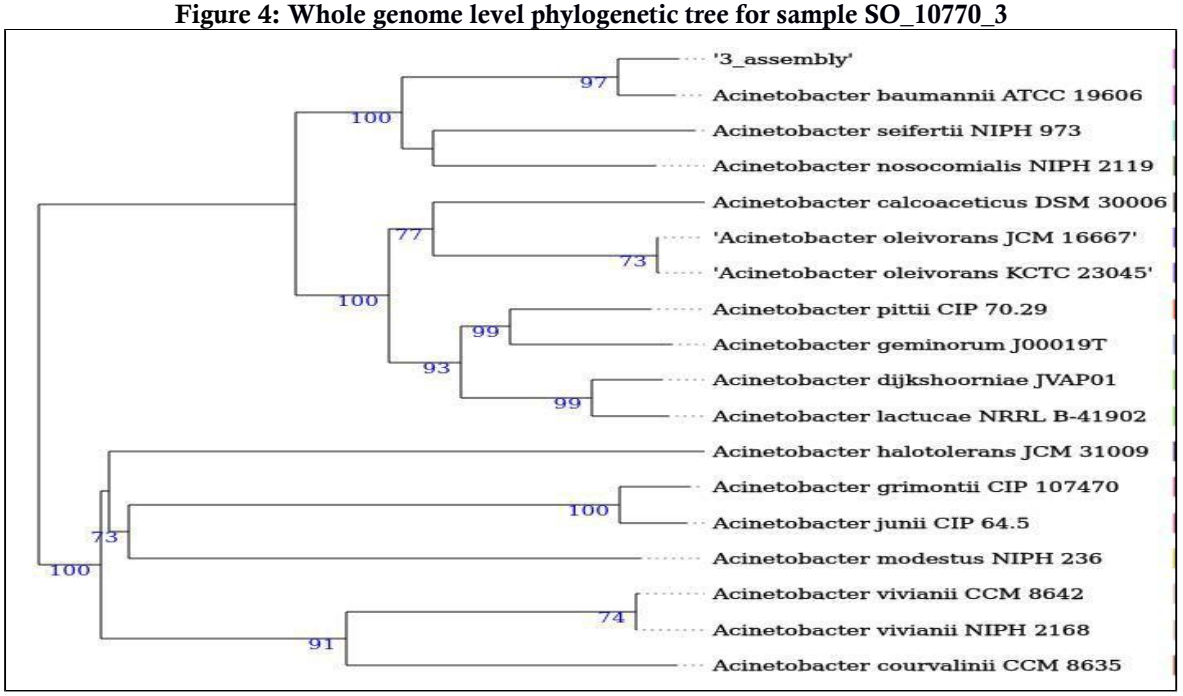
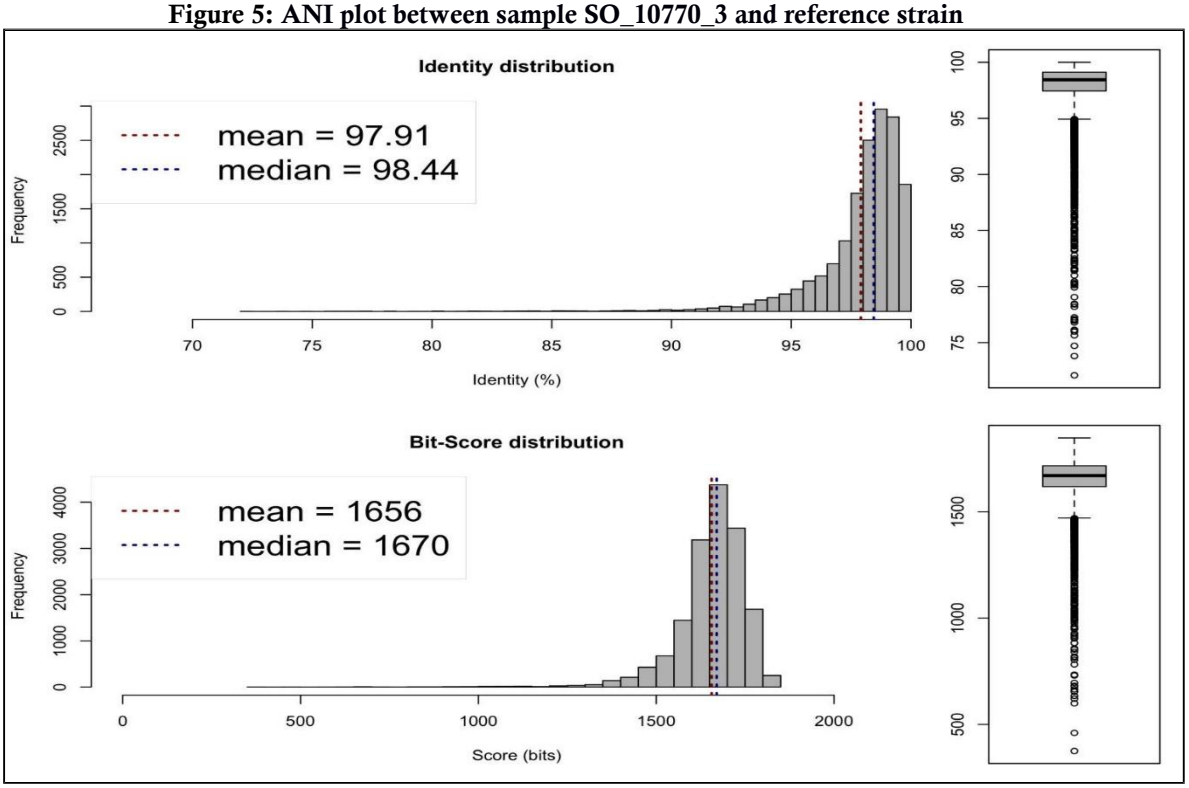


Figure 5: ANI plot between sample SO_10770_3 and reference strain



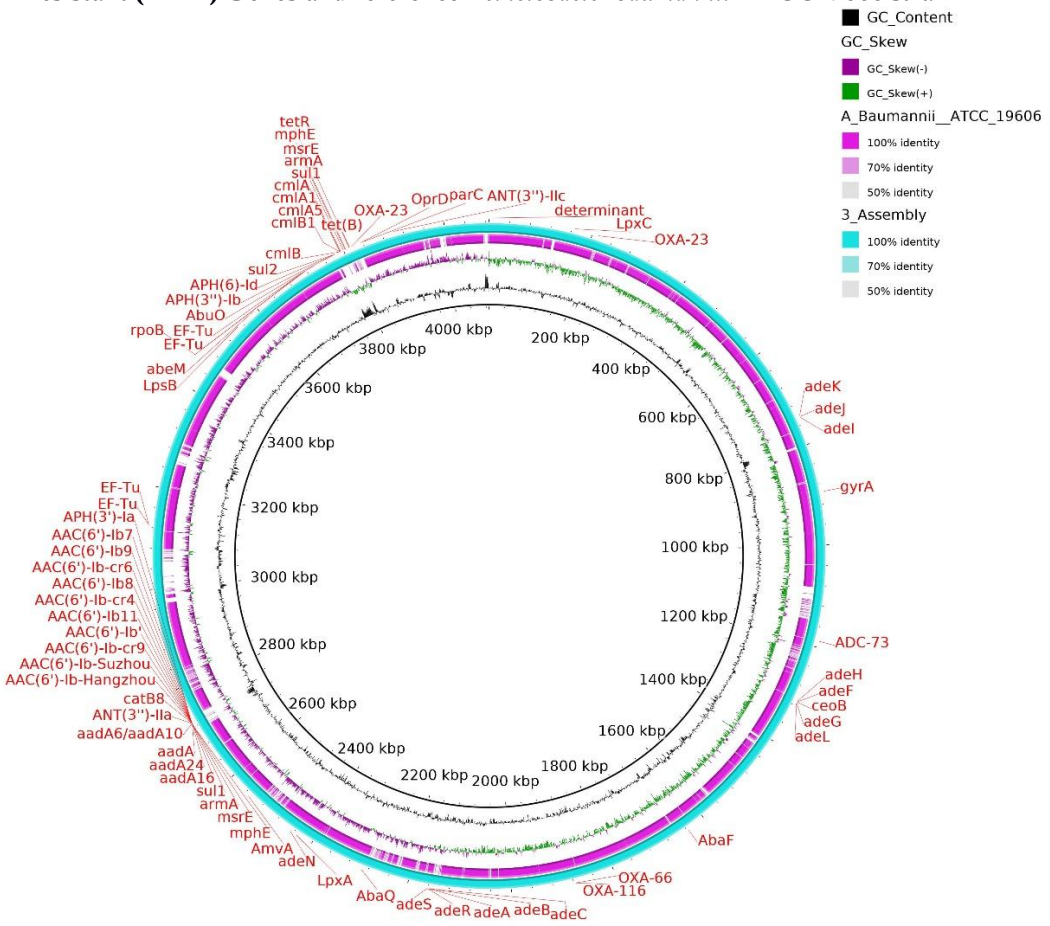
The high ANI value (>95%) confirms species identity, while phylogenetic clustering supports genomic relatedness to established reference strains. Long-read assembly ensures reliable genome-wide similarity estimation by minimizing assembly fragmentation bias.

3.5 Comparative Genome Analysis

Comparative genome visualization using BRIG demonstrated high genomic similarity between SO_10770_3 and *A. baumannii* ATCC19606. The isolate demonstrated 97.91% ANI and 81.5% d4 relatedness to *A. baumannii*

ATCC19606, confirming species-level identity (Figure 6).
Regions of divergence corresponded to resistance islands and mobile genetic elements.

Figure 6: BRIG Circular genome comparison image of sample SO_10770_3 plotted with Anti Microbial Resistant (AMR) Genes and reference *Acinetobacter baumannii* ATCC19606 strain



The divergence hotspots identified in the circular comparison are consistent with horizontally acquired resistance islands and plasmid integrations, supporting structural evolution under antibiotic pressure.

3.6 Antimicrobial Resistance Genes and Associated Mobile Genetic Elements

CARD-based analysis identified 395 AMR-related hits, including:

- RND efflux pump systems
- gyrA-associated fluoroquinolone resistance
- Carbapenem resistance genes
- Tetracycline resistance determinants
- Rifamycin resistance-associated RNA polymerase mutations

To evaluate mobility potential, 500 bp flanking regions of AMR genes were screened against the ACLAME database:

- 55 upstream MGE hits
- 165 downstream MGE hits

The high number of mobile genetic elements flanking AMR genes suggests substantial horizontal gene transfer potential. Long-read sequencing enables accurate localization of these MGEs relative to resistance genes, which is critical for understanding dissemination risk.

3.7 Virulence Factors and Mobility Analysis

A total of 341 virulence-associated proteins were identified using VFDB (≥70% identity threshold). These included

genes related to:

- Biofilm formation
- Capsular polysaccharide biosynthesis
- Iron acquisition systems
- Efflux pumps

MGE screening around virulence genes revealed:

- 1 upstream MGE hit
- 0 downstream MGE hits

Unlike AMR genes, virulence determinants showed limited association with mobile elements, suggesting relative genomic stability. This distinction indicates that antimicrobial resistance determinants are under stronger horizontal selection pressure compared to virulence genes.

DISCUSSION

Structural Evolution of Carbapenem Resistance

The long-read genome assembly of Acinetobacter baumannii SO_10770_3 provides high-resolution insight into the structural evolution of carbapenem resistance within a ~4 Mb genome architecture comprising a large chromosomal contig and two plasmid contigs. The identification of 395 antimicrobial resistance–associated hits, many of which were flanked by mobile genetic elements, underscores the role of genomic plasticity in shaping the extensively drug-resistant (XDR) phenotype. Carbapenem resistance in A. baumannii is predominantly mediated by class D carbapenem-hydrolyzing β-lactamases (CHDLs), particularly OXA-type enzymes such as OXA-23, OXA-24/40, OXA-51-like, and OXA-58 (Poirel & Nordmann, 2006; Evans & Amyes, 2014). In the present study, structural localization of OXA genes within resistance-associated regions confirms that carbapenem resistance is embedded within mobile genomic frameworks rather than isolated mutational events.

The detection of multiple resistance-associated regions and insertion sequences suggests ongoing structural remodeling. Resistance islands in A. baumannii, often described as AbaR-type islands, are known to integrate into conserved chromosomal loci and accumulate diverse resistance determinants over time (Fournier et al., 2006). The presence of numerous mobile genetic element (MGE) hits—55 upstream and 165 downstream of AMR genes—strongly indicates active recombination and transposition events contributing to the expansion of the resistome. Such clustering of AMR genes within mobile frameworks reflects adaptive genome restructuring under antibiotic selection pressure, facilitating the transition from multidrug-resistant (MDR) to extensively drug-resistant phenotypes.

Insertion sequence–mediated promoter activation represents a key mechanism driving elevated carbapenem resistance. ISAba1 and ISAba3 are particularly associated with enhanced expression of OXA genes (Turton et al., 2006). The structural proximity of IS elements to carbapenemase loci in this genome supports transcriptional upregulation mechanisms rather than mere gene acquisition. This genomic configuration suggests evolutionary fine-tuning for sustained carbapenem resistance, particularly in high-antibiotic-pressure clinical environments such as intensive care units.

Mobility Potential of OXA Genes

One of the most significant findings of this study is the structural differentiation between chromosomal and plasmid-borne resistance determinants. The assembly resolved two plasmid contigs in addition to the chromosome, enabling precise localization of resistance genes. The plasmid association of OXA-58, in particular, highlights its mobility potential. OXA-58 is frequently reported as plasmid-encoded and flanked by ISAba3-like elements forming composite transposons (Poirel et al., 2005; Hamidian & Hall, 2018). This arrangement enhances both gene expression and horizontal transfer capacity.

Plasmids identified in this genome likely function as multidrug resistance platforms, as evidenced by the presence of multiple AMR determinants within mobile contexts. The high frequency of downstream MGE hits (165) suggests that resistance genes are embedded within transposon-rich environments, increasing the likelihood of mobilization across replicons. Such structural organization promotes inter-strain dissemination and may contribute to clonal expansion within hospital settings.

The coexistence of chromosomal carbapenemase genes alongside plasmid-borne OXA determinants may provide a dual evolutionary advantage. Chromosomal integration ensures stable vertical inheritance, while plasmid carriage facilitates horizontal spread. This redundancy may sustain elevated carbapenem minimum inhibitory concentrations (MICs) and reduce the efficacy of last-resort β-lactams. From an evolutionary perspective, this dual localization strategy enhances both persistence and transmissibility, accelerating dissemination across clinical populations.

Horizontal Gene Transfer Implications

Horizontal gene transfer (HGT) plays a central role

in the evolution of XDR A. baumannii. The identification of 220 total MGE hits flanking AMR genes (combined upstream and downstream) provides strong evidence for ongoing or historical gene mobilization events. Conjugative plasmids remain the principal vehicles for carbapenemase dissemination, although natural transformation and bacteriophage-mediated transduction may also contribute (Touchon et al., 2014).

The presence of prophage regions within the genome further supports genome diversification through recombination mechanisms. Although prophages were not directly associated with AMR clusters in this dataset, their presence indicates genomic instability that may facilitate structural rearrangements. In contrast, virulence factors (341 identified) showed minimal MGE association (only one upstream hit), suggesting that resistance determinants are under stronger horizontal selection pressure than virulence genes. This differential mobility pattern implies that antibiotic exposure, rather than host adaptation alone, is the primary driver of recent genomic restructuring.

The high average nucleotide identity (97.91%) with A. baumannii ATCC19606 confirms species-level identity, while divergence regions observed in comparative genome analysis correspond primarily to resistance-associated loci. These divergence hotspots likely represent horizontally acquired segments stabilized within the genome. The rapid global dissemination of carbapenem-resistant A. baumannii, classified by the World Health Organization as a critical-priority pathogen, can be attributed to precisely such mobile and recombinogenic genomic features (Tacconelli et al., 2018).

Advantages of Long-Read Sequencing in Structural Genomics

A major strength of this study lies in the application of long-read sequencing technology developed by Oxford Nanopore Technologies, which enabled structural resolution of chromosomal and plasmid elements within a minimal three-contig assembly. Unlike short-read sequencing, which often produces fragmented assemblies due to repetitive IS elements and transposons, long-read sequencing spans these repetitive regions, enabling accurate structural mapping (Wick et al., 2017).

In this study, long reads allowed- Precise separation of chromosome and plasmids; Accurate localization of AMR genes relative to MGEs; Identification of structural divergence regions; Reliable detection of plasmid-associated carbapenemase genes

Short-read assemblies frequently collapse repetitive IS elements or misassign plasmid sequences to chromosomal contigs, limiting structural interpretation. Long-read sequencing overcomes these challenges, providing contiguous assemblies that preserve genomic architecture. Although long-read platforms historically exhibit higher per-base error rates, iterative polishing significantly improves consensus accuracy (Vaser et al., 2017), making them highly reliable for structural genomic studies.

The ability to resolve AMR genes within their precise genomic context is critical for epidemiological tracking, risk assessment of horizontal dissemination, and prediction of outbreak dynamics. As antimicrobial resistance surveillance increasingly relies on genome-based approaches, long-read sequencing offers superior resolution for understanding the structural determinants underlying XDR phenotypes.

The Nanopore-based genome assembly of A. baumannii SO_10770_3 reveals a structurally dynamic resistome characterized by clustered AMR genes, extensive mobile genetic elements, and plasmid-mediated carbapenemase dissemination. The high density of MGEs flanking resistance determinants underscores the central role of horizontal gene transfer in resistance evolution. By resolving chromosomal and plasmid architecture within a near-complete assembly, this study highlights the transformative value of long-read sequencing in deciphering the genomic mechanisms driving carbapenem resistance and XDR emergence.

References

1. Alcock, B. P., Raphenya, A. R., Lau, T. T. Y., Tsang, K. K., Bouchard, M., Edalatmand, A., … McArthur, A. G. (2020). CARD 2020: Antibiotic resistome surveillance with the Comprehensive Antibiotic Resistance Database. Nucleic Acids Research, 48(D1), D517–D525. https://doi.org/10.1093/nar/gkz935
2. Antunes, L. C. S., Visca, P., & Towner, K. J. (2014). Acinetobacter baumannii: Evolution of a global pathogen. Nature Reviews Microbiology, 12(8), 537–548. https://doi.org/10.1038/nrmicro3298
3. Arndt, D., Grant, J. R., Marcu, A., Sajed, T., Pon, A., Liang, Y., & Wishart, D. S. (2016). PHASTER: A better, faster version of the PHAST phage search tool. Nucleic Acids Research, 44(W1), W16–W21. https://doi.org/10.1093/nar/gkw387
4. Arredondo-Alonso, S., Willems, R. J., van Schaik, W., & Schürch, A. C. (2017). On the (im)possibility of reconstructing plasmids from short-read whole-genome sequencing data. Microbial Genomics, 3(10), e000128. https://doi.org/10.1099/mgen.0.000128
5. Bertelli, C., Laird, M. R., Williams, K. P., Simon Fraser University Research Computing Group, Lau, B. Y., Hoad, G., … Brinkman, F.

S. L. (2017). IslandViewer 4: Expanded prediction of genomic islands for larger-scale datasets. Nucleic Acids Research, 45(W1), W30–W35. https://doi.org/10.1093/nar/gkx343
6. Clinical and Laboratory Standards Institute. (2023). Performance standards for antimicrobial susceptibility testing (33rd ed.). CLSI supplement M100. CLSI.
7. De Coster, W., D'Hert, S., Schultz, D. T., Cruts, M., & Van Broeckhoven, C. (2018). NanoPack: Visualizing and processing long-read sequencing data. Bioinformatics, 34(15), 2666–2669. https://doi.org/10.1093/bioinformatics/bty149
8. Diancourt, L., Passet, V., Nemec, A., Dijkshoorn, L., & Brisse, S. (2010). The population structure of Acinetobacter baumannii: Expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS ONE, 5(4), e10034. https://doi.org/10.1371/journal.pone.0010034
9. Evans, B. A., & Amyes, S. G. B. (2014). OXA β-lactamases. Clinical Microbiology Reviews, 27(2), 241–263. https://doi.org/10.1128/CMR.00117-13
10. Fournier, P. E., Vallenet, D., Barbe, V., Audic, S., Ogata, H., Poirel, L., … Raoult, D. (2006). Comparative genomics of multidrug resistance in Acinetobacter baumannii. PLoS Genetics, 2(1), e7. https://doi.org/10.1371/journal.pgen.0020007
11. Hamidian, M., & Hall, R. M. (2018). The AbaR antibiotic resistance islands found in Acinetobacter baumannii global clone 1. Antimicrobial Agents and Chemotherapy, 62(8), e01707-17. https://doi.org/10.1128/AAC.01707-17
12. Jain, M., Olsen, H. E., Paten, B., & Akeson, M. (2016). The Oxford Nanopore MinION: Delivery of nanopore sequencing to the genomics community. Nature Methods, 13(4), 351–356. https://doi.org/10.1038/nmeth.3797
13. Kolmogorov, M., Yuan, J., Lin, Y., & Pevzner, P. A. (2019). Assembly of long, error-prone reads using repeat graphs. Nature Biotechnology, 37(5), 540–546. https://doi.org/10.1038/s41587-019-0072-8
14. Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., … Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions. Clinical Microbiology and Infection, 18(3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x
15. Peleg, A. Y., Seifert, H., & Paterson, D. L. (2008). Acinetobacter baumannii: Emergence of a successful pathogen. Clinical Microbiology Reviews, 21(3), 538–582. https://doi.org/10.1128/CMR.00058-07
16. Poirel, L., & Nordmann, P. (2006). Carbapenem resistance in Acinetobacter baumannii: Mechanisms and epidemiology. Clinical Microbiology and Infection, 12(9), 826–836. https://doi.org/10.1111/j.1469-0691.2006.01456.x
17. Poirel, L., Marqué, S., Héritier, C., Segonds, C., Chabanon, G., & Nordmann, P. (2005). OXA-58, a novel class D β-lactamase involved in resistance to carbapenems in Acinetobacter baumannii. Antimicrobial Agents and Chemotherapy, 49(1), 202–208. https://doi.org/10.1128/AAC.49.1.202-208.2005
18. Robertson, J., & Nash, J. H. E. (2018). MOB-suite: Software tools for clustering, reconstruction and typing of plasmids from draft assemblies. Microbial Genomics, 4(8), e000206. https://doi.org/10.1099/mgen.0.000206
19. Seemann, T. (2014). Prokka: Rapid prokaryotic genome annotation. Bioinformatics, 30(14), 2068–2069. https://doi.org/10.1093/bioinformatics/btu153
20. Siguier, P., Perochon, J., Lestrade, L., Mahillon, J., & Chandler, M. (2006). ISfinder: The reference centre for bacterial insertion sequences. Nucleic Acids Research, 34(Database issue), D32–D36. https://doi.org/10.1093/nar/gkj014
21. Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., … WHO Pathogens Priority List Working Group. (2018). Discovery, research, and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria. The Lancet Infectious Diseases, 18(3), 318–327. https://doi.org/10.1016/S1473-3099(17)30753-3
22. Touchon, M., Cury, J., Yoon, E. J., Krizova, L., Cerqueira, G. C., Murphy, C., … Rocha, E. P. C. (2014). The genomic diversification of the whole Acinetobacter genus: Origins, mechanisms, and consequences. Genome Biology and Evolution, 6(10), 2866–2882. https://doi.org/10.1093/gbe/evu225
23. Turton, J. F., Ward, M. E., Woodford, N., Kaufmann, M. E., Pike, R., Livermore, D. M., & Pitt, T. L. (2006). The role of ISAba1 in expression of OXA carbapenemase genes in Acinetobacter baumannii. Antimicrobial Agents and Chemotherapy, 50(2), 384–390. https://doi.org/10.1128/AAC.50.2.384-390.2006
24. Vaser, R., Sović, I., Nagarajan, N., & Šikić, M. (2017). Fast and accurate de novo genome assembly from long uncorrected reads. Genome Research, 27(5), 737–746. https://doi.org/10.1101/gr.214270.116
25. Wick, R. R., Judd, L. M., & Holt, K. E. (2017). Unicycler: Resolving bacterial genome

assemblies from short and long sequencing reads. PLoS Computational Biology, 13(6), e1005595. https://doi.org/10.1371/journal.pcbi.1005595

