Research Square

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Molecular Characterization of Colistin Resistance Determinants in Clinical and Patient-Environmental Multidrug-Resistant *Acinetobacter baumannii*

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Abstract

Background

Acinetobacter baumannii has emerged as an important nosocomial pathogen due to its multidrug resistance (MDR) and increasing resistance to colistin, one of the last-line therapeutic agents. Understanding the molecular mechanisms underlying colistin resistance is essential for effective antimicrobial stewardship and infection control.

Methods

A prospective hospital-based study was conducted between August 2023 and October 2024 and included 110 MDR *A. baumannii* isolates comprising 55 clinical and 55 environmental isolates recovered from patient surroundings. Colistin susceptibility was determined by broth microdilution (BMD) according to Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines. Whole-genome sequencing was performed using the Illumina MiSeq platform. Genomic analyses were undertaken to identify plasmid-mediated *mcr* genes and chromosomal mutations associated with colistin resistance.

Results

Of the 55 clinical isolates, 43 (78.2%) were colistin susceptible and 12 (21.8%) were resistant, whereas 48 (87.3%) environmental isolates were susceptible and 7 (12.7%) were resistant. Most isolates exhibited a colistin minimum inhibitory concentration (MIC) of 1 mg/L. Plasmid-mediated *mcr* genes were identified in three clinical resistant isolates (*mcr-1*, *n* = 2; *mcr-4*, *n* = 1) and one environmental resistant isolate (*mcr-1*, *n* = 1). Chromosomal resistance mechanisms among clinical isolates included mutations in *pmrA* (M12I), *pmrB* (A138T and A444V), *lpxD* (E117K), and insertion of ISAba1 upstream of *eptA/pmrC*. A *pmrB* (T235I) mutation was detected in one environmental isolate. Five colistin-resistant isolates lacked identifiable plasmid-mediated or chromosomal resistance determinants, suggesting the involvement of alternative resistance mechanisms, including heteroresistance.

Conclusions

Colistin resistance among MDR *A. baumannii* was predominantly associated with chromosomal mutations rather than plasmid-mediated *mcr* genes. The detection of resistant isolates lacking known genetic determinants highlights the complexity of colistin resistance and suggests the presence of additional resistance mechanisms. Integrating phenotypic susceptibility testing with whole-genome sequencing can improve the detection and surveillance of colistin resistance and support antimicrobial stewardship and infection prevention strategies.

Introduction

Acinetobacter baumannii is a major opportunistic pathogen responsible for a wide range of healthcare-associated infections, including ventilator-associated pneumonia, bloodstream infections, urinary tract infections, surgical site infections, and wound infections, particularly among critically ill and immunocompromised patients. [1, 2] It's remarkable ability to survive under adverse environmental conditions and persist on hospital surfaces facilitates prolonged transmission within healthcare settings, contributing to outbreaks and making infection control particularly challenging.[3] The increasing prevalence of multidrug-resistant (MDR) *A. baumannii* has further complicated the management of these infections and has been recognized as a global public health concern. [4]

The widespread emergence of MDR *A. baumannii* has markedly reduced the effectiveness of commonly used antimicrobial agents, including carbapenems, aminoglycosides, and fluoroquinolones, leaving colistin (polymyxin E) as one of the few remaining therapeutic options. However, increasing reports of colistin resistance have raised serious concerns regarding the treatment of infections caused by extensively drug-resistant *A. baumannii*. [5, 6] Colistin exerts its bactericidal activity by binding to the lipid A component of lipopolysaccharide in the outer membrane, resulting in membrane disruption and bacterial cell death.[6]

Colistin resistance in *A. baumannii* is predominantly mediated by chromosomal alterations that modify the lipid A target. These include mutations in the two-component regulatory system genes *pmrA* and *pmrB* and in the lipid A biosynthesis genes *lpxA*, *lpxC*, and *lpxD*. Overexpression of the phosphoethanolamine transferase encoded by *eptA/pmrC*, often associated with the insertion sequence ISAba1, further contributes to lipid A modification and reduced colistin susceptibility. In contrast, plasmid-mediated *mcr* genes, which are widely distributed among Enterobacterales, have only occasionally been reported in *A. baumannii* but remain of considerable concern because of their potential for horizontal gene transfer. [4–8]

Whole-genome sequencing (WGS) has become an invaluable tool for investigating antimicrobial resistance by enabling comprehensive identification of acquired resistance genes and chromosomal mutations at high resolution. When integrated with phenotypic susceptibility testing, WGS provides a robust approach for elucidating resistance mechanisms and supports surveillance of emerging resistant clones within healthcare settings. [9] Although several studies have investigated colistin resistance in clinical *A. baumannii* isolates, comparative genomic data describing resistance determinants among both clinical and hospital environmental isolates remain limited, particularly in India. [10]

Hospital environments serve as important reservoirs for MDR *A. baumannii*, with contaminated surfaces contributing to the persistence and transmission of resistant strains. Simultaneous investigation of clinical and environmental isolates is therefore essential to better understand the epidemiology of colistin resistance and to guide effective infection prevention and control strategies. [9, 10]

In this study, we determined the phenotypic susceptibility of clinical and environmental MDR *A. baumannii* isolates to colistin using the broth microdilution method and characterized plasmid-mediated *mcr* genes and chromosomal resistance determinants using whole-genome sequencing. We further compared the distribution of these resistance mechanisms between clinical and environmental isolates to improve the understanding of the molecular epidemiology of colistin-resistant *A. baumannii* in a tertiary care hospital.

Materials and methods

Study design and bacterial isolates:

A prospective hospital-based study was conducted in the Department of Microbiology, JSS Medical College and Hospital, Mysuru, India, between August 2023 and October 2024. A total of 110 multidrug-resistant (MDR) *Acinetobacter baumannii* isolates were included, comprising 55 clinical isolates and 55 environmental isolates recovered from the surroundings of patients with MDR *A. baumannii* infection. Repeat isolates obtained from the same patient or environmental site were excluded from the study.

Sample collection and bacterial identification:

Clinical specimens, including blood, urine, sputum, pus, endotracheal aspirates, and other body fluids, were processed according to standard microbiological procedures. [11] Specimens were cultured on MacConkey agar and 5% sheep blood agar (HiMedia Laboratories, Mumbai, India), while urine samples were additionally cultured on HiCrome UTI agar (HiMedia Laboratories, Mumbai, India). Semi-quantitative culture was performed for urine and endotracheal aspirate specimens.

Environmental surveillance was conducted by collecting samples from patient surroundings following the isolation of MDR *A. baumannii* from clinical specimens. Five environmental samples were collected from each patient area, including bed rails, beds, trolleys, floors, walls, and bathrooms. Environmental specimens were cultured on MacConkey agar and 5% sheep blood agar and incubated aerobically at 37°C for 18–24 h.

Presumptive *Acinetobacter* isolates were identified based on colony morphology, Gram staining, and conventional phenotypic characteristics, including catalase positivity, oxidase negativity, and non-motility. Clinical isolates were identified using the VITEK® 2 automated identification system (bioMérieux, Marcy-l'Étoile, France), whereas environmental isolates were identified using standard biochemical tests, including indole production, urease activity, citrate utilization, and triple sugar iron (TSI) agar reactions.

Antimicrobial susceptibility testing:

Antimicrobial susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) 2024 [12] guidelines. Clinical isolates were tested using the VITEK® 2 automated system

with AST-N406 cards (bioMérieux, Marcy-l'Étoile, France), whereas environmental isolates were tested using the Kirby–Bauer disk diffusion method [14].

Determination of colistin minimum inhibitory concentration:

Colistin minimum inhibitory concentrations (MICs) were determined by the broth microdilution (BMD) method [15] using the commercially available MIKROLATEST colistin MIC kit (Erba Lachema, Czech Republic) containing cation-adjusted Mueller–Hinton broth and serial two-fold dilutions of colistin. Bacterial inocula equivalent to a 0.5 McFarland standard were prepared, and testing was performed according to the manufacturer's instructions and CLSI 2024 [12] recommendations. MICs were interpreted according to CLSI/EUCAST 2024 [12, 13] breakpoints, where isolates with MICs ≤ 2 µg/mL were considered susceptible and those with MICs > 2 µg/mL were considered resistant. Quality control was performed using *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 in accordance with CLSI and EUCAST [12, 13] recommendations.

Whole-genome sequencing and analysis:

Genomic DNA was extracted from 110 multidrug-resistant (MDR) *Acinetobacter baumannii* isolates using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). DNA was quantified using a Qubit fluorometer with the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing libraries with a 450 bp insert size were prepared using the Ultra FS II DNA Library Preparation Kit (New England Biolabs, Hitchin, UK). Library quality was assessed using an Agilent TapeStation (Agilent Technologies, Santa Clara, CA, USA), and paired-end sequencing was performed on the Illumina MiSeq platform (Illumina, San Diego, CA, USA).

Raw sequencing reads were assessed for quality using FastQC [16] and assembled de novo using SPAdes v3.14 [17]. Genome annotation was performed using Prokka v1.5 [18]. Assembly quality was evaluated using Confindr [19], MultiQC [20], and Qualifyr, [21] and genomes meeting the predefined quality criteria were included for downstream analyses.

Identification of colistin resistance determinants:

The assembled genomes were analysed using AMRFinderPlus [22] v3.12.8 with the *Acinetobacter* reference database to identify plasmid-mediated colistin resistance genes (*mcr-1* to *mcr-10*) and chromosomal determinants associated with colistin resistance. Mutations in the two-component regulatory system genes (*pmrA* and *pmrB*), lipid A biosynthesis genes (*lpxA*, *lpxC*, and *lpxD*), and the phosphoethanolamine transferase gene (*eptA/pmrC*) were investigated. The presence of the insertion sequence ISAba1 upstream of *eptA/pmrC* was also assessed as a potential mechanism of gene overexpression. The identified genetic determinants were correlated with phenotypic colistin minimum inhibitory concentration (MIC) results obtained by broth microdilution according to the Clinical and Laboratory Standards Institute (CLSI) 2024 [12] guidelines.

Results

A total of 110 MDR *A. baumannii* isolates were included, comprising 55 clinical and 55 environmental isolates collected between August 2023 and October 2024 collected from a tertiary care hospital. Clinical isolates were recovered from blood, urine, sputum, pus, endotracheal aspirates, and other body fluids, whereas environmental isolates were obtained from patient surroundings. Among the 275 environmental samples collected, 67 MDR *Acinetobacter* isolates were recovered, of which 55 non-duplicate isolates were included for further analysis.

Colistin susceptibility among clinical and environmental isolates:

Colistin susceptibility was determined by the broth microdilution (BMD) method according to CLSI 2024 guidelines.

Among the clinical isolates, 43/55 (78.2%) were colistin susceptible, whereas 12/55 (21.8%) were resistant. The majority of clinical isolates (26/55; 47.3%) exhibited a minimum inhibitory concentration (MIC) of 1 mg/L. Among the environmental isolates, 48/55 (87.3%) were susceptible and 7/55 (12.7%) were resistant, with most isolates (36/55; 65.5%) demonstrating an MIC of 1 mg/L. The distribution of colistin MIC values among clinical and environmental isolates is presented in (Table 1**)**.

Table 1
Distribution of colistin MIC values determined by broth microdilution (BMD) among clinical and environmental *Acinetobacter baumannii* isolates

Colistin MIC (mg/L)	**CLSI (2024) Interpretation**	**Clinical isolates, n (%)**	**Environmental isolates, n (%)**
0.5	Susceptible	12 (21.8)	3 (5.5)
1	Susceptible	26 (47.3)	36 (65.5)
2	Susceptible	5 (9.1)	9 (16.4)
4	Resistant	12 (21.8)	7 (12.7)
Total		**55**	**55**

Detection of plasmid-mediated mcr genes:

Among the 12 colistin-resistant clinical isolates, plasmid-mediated *mcr* genes were detected in three isolates. The *mcr-1* gene was identified in two isolates (16.7%), whereas *mcr-4* was detected in one isolate (8.3%). Among the seven colistin-resistant environmental isolates, *mcr-1* was identified in one isolate (14.3%), while *mcr-4* was not detected. The distribution of plasmid-mediated *mcr* genes is summarized in (Table 2**)**.

Table 2
Distribution of plasmid-mediated *mcr* genes among colistin resistant *Acinetobacter baumannii* isolates.

Gene	Clinical isolates (n = 12), n(%)	Environmental isolates (n = 7), n(%)
mcr-1	2 (16.7)	1 (14.3)
mcr-4	1 (8.3)	0
Total positive isolates	**3 (25.0)**	**1 (14.3)**

Footnote: *Some isolates carried different mcr genes; total positives represent isolates positive for at least one mcr gene.*

Chromosomal determinants associated with colistin resistance:

Chromosomal alterations associated with colistin resistance were identified predominantly among the clinical isolates, whereas only a single mutation was detected among the environmental isolates.

Mutations involving the pmrAB two-component regulatory system included *pmrA* M12I (2/12; 16.7%), *pmrB* A138T (2/12; 16.7%), and *pmrB* A444V (2/12; 16.7%). Mutations in *lpxD* (E117K) were identified in two clinical isolates (16.7%). In addition, insertion of ISAba1 upstream of the *eptA/pmrC* locus was detected in one clinical isolate (8.3%).

Among the environmental isolates, a single nonsynonymous mutation in *pmrB* (T235I) was identified in one of seven colistin-resistant isolates (14.3%). No alterations in *lpxD* or ISAba1 insertion upstream of *eptA/pmrC* were detected among the remaining environmental isolates. The chromosomal resistance determinants identified in clinical and environmental isolates are presented in (Table 3**)**.

Table 3
Chromosomal alterations identified among colistin-resistant *Acinetobacter baumannii* isolates.

Isolate source	Gene	Isolates with alteration, n (%)	Nucleotide change	Amino acid change
Clinical	*pmrA*	2/12 (16.7)	35A > G	M12I
Clinical	*pmrB*	2/12 (16.7)	412G > A	A138T
Clinical	*pmrB*	2/12 (16.7)	1331C > T	A444V
Clinical	*lpxD*	2/12 (16.7)	349G > A	E117K
Clinical	*eptA/pmrC* promoter	1/12 (8.3)	ISAba1 insertion	Promoter activation
Environmental	*pmrB*	1/7 (14.3)	704C > T	T235I

Correlation of phenotypic and genotypic colistin resistance:

Among the seven colistin-resistant environmental isolates, one isolate harboured the plasmid-mediated *mcr-1* gene and one isolate possessed a *pmrB* (T235I) mutation. Five colistin-resistant environmental isolates lacked detectable plasmid-mediated *mcr* genes or the chromosomal alterations investigated, suggesting the presence of additional mechanisms Oof colistin resistance.

Overall, chromosomal resistance-associated alterations were more frequently identified than plasmid-mediated *mcr* genes among the colistin-resistant isolates, particularly in the clinical isolates. The isolate-level distribution of plasmid-mediated genes and chromosomal mutations is presented in **(Supplementary Table S1).**

Discussion

The present study provides a comprehensive phenotypic and genomic characterization of colistin resistance among multidrug-resistant (*MDR*) *Acinetobacter baumannii* isolated from both clinical and patient-environmental sources in a tertiary care hospital. By integrating broth microdilution susceptibility testing with whole-genome sequencing, this study identified both plasmid-mediated and chromosomal mechanisms contributing to colistin resistance. Clinical isolates demonstrated a higher prevalence of colistin resistance than environmental isolates, with resistance largely mediated by chromosomal alterations involving the **pmrAB** two-component regulatory system, **lpxD**, and **ISAba1-associated eptA/pmrC** activation, whereas plasmid-mediated **mcr** genes were detected only in a small proportion of isolates. Additionally, several phenotypically resistant isolates lacked recognized resistance determinants, suggesting the presence of alternative mechanisms such as heteroresistance, regulatory alterations, or novel mutations.

Detection of colistin resistance by Microbroth dilution (MBD) reference method :

In the present study, among the 55 clinical isolates, 43 isolates (78.19%) were sensitive and 12 isolates (21.81%) were resistant to colistin by Microbroth Dilution (MBD) method.

These findings aligned with the studies by *Al-Kadmy et al.* (Iraq, 2021) [(23)], *Masalmeh et al.* (Cyprus, 2024) [(24)] *and Abbas et.al.* (Iraq, 2025) [(25)] who have reported 21.5%, 21% and 29.7% colistin resistance respectively among clinical isolates of MDR *A. baumanii.*

In contrast, a study by *Luccas D et al.* (2021) [(26)] from Japan, reported 6.5% colistin resistance by Microbroth dilution method. This contrast likely reflects the restricted use of colistin in different geographical region.

Among the 55 environmental isolates, 48 isolates (48/55; 87.28%) were sensitive and 7 isolates (7/55; 12.72%) were resistant to colistin by Microbroth dilution (MBD) reference method.

Environmental isolates in the present study showed a lower resistance rate 7 (12.72%) compared to the clinical isolates (12/55; 21.81%).

Similar findings have been reported in a study by *Sacco et al*. (Italy, 2021) [27] *Zafer et al. (Egypt, 2023)* [28] and *Akilli et al. (Turkey, 2025)*[29], who has reported 10.7%, 10% and 11.8% colistin resistance among environmental isolates.

Overall, the lower rate of colistin resistance observed in the present study is consistent with the colistin resistance rates geographically [23–29].

Plasmid-mediated mcr genes & chromosomal-mediated mutations associated with colistin resistance:

In the present study, clinical MDR *A. baumannii* exhibited a combination of colistin resistance mechanism, involving both plasmid-mediated *mcr* genes and chromosomal mediated modifications, whereas environmental MDR *A. baumanii* demonstrated a more limited resistance profile, with a single chromosomal *pmrB* substitution and lower detection of plasmid-mediated *mcr* genes. Mutations identified in *pmrA*, *pmrB*, and *lpxD*, together with ISAba1 insertion upstream of *eptA/pmrC*, have previously been associated with lipid A modification and reduced susceptibility to colistin [8–10].

The plasmid-mediated *mcr* genes were higher among clinical isolates (n = 3) compared to the environmental isolates (n = 1), *mcr*-1 detected in both clinical (2/12, 16.7%) and environmental isolates (1/7, 14.3%**)** and *mcr*-4 gene being detected in only one clinical isolate **(**1/12, 8.3%).

Chromosomal alterations were identified among 9 (9/12; 75%) isolates in the *pmrAB* two-component regulatory system, including *pmrB A138T, pmrB A444V, and pmrA M12I* among 2/12 (16.6%) colistin resistant clinical isolates. In addition, a non-synonymous substitution (E117K) was detected in *lpxD*, a gene involved in lipid A biosynthesis among 2/12 (16.6%) colistin resistant clinical isolates. Additionally, ISAba1 upstream of the *eptA/pmrC* locus was observed in 1/12 (8.3%) clinical isolates. This IS-associated mechanism was observed alongside *pmrAB* mutations, suggesting a combined mechanism to colistin resistance among clinical isolates

These findings aligned with a study by *Shiromani et al.* (2026,India) [30] who has reported 5 (11%) *mcr1* gene among clinical isolates and identified ISAba1 upstream of the *eptA/pmrC* locus with chromosomal substitutions like *pmrA M12I*, *pmrB A138T/A444V*, and *lpxD E117K* in 3/5 (60%) colistin-resistant clinical isolates.

Similar chromosomal alterations have been described in a study by *Gerson et al.* (2019)[8] (Germany) who has demonstrated ISAba1 associated overexpression of *eptA* in 7/27 (25.9%) among colistin-resistant *clinical* isolates, supporting that IS-mediated dysregulation of lipid A modification pathways represents a recurring mechanism across geographical regions.

In contrast, the chromosomal mutations observed among the environmental isolates in the present study was by a single *pmrB T235I* substitution without the IS-mediated *eptA* modification which was observed in only one isolate (1/7; 14.28%), which was similar in a study by *Pragasam et al.*, (2017,India) [9] who has reported *pmrAB* mutations alone which contributed to reduced colistin susceptibility among 2 isolates (2/26; 7.69%).

Together, these observations indicates that similar resistance mechanisms to colistin are emerging across different geographical regions [8, 10].

Phenotype–genotype discordance:

Despite the identification of known plasmid-mediated and chromosomal resistance determinants, several phenotypically colistin-resistant isolates lacked recognised genetic mechanisms of resistance. Similar phenotype–genotype discordance has been reported in previous studies and may be attributed to heteroresistance [8, 10], mutations in regulatory regions not captured by routine genomic analysis, altered gene expression, epigenetic regulation, or as-yet unidentified resistance mechanisms. These findings highlight the complexity of colistin resistance in *A. baumannii* and emphasise that whole-genome sequencing should complement rather than replace phenotypic susceptibility testing for accurate detection of colistin resistance.[10, 12, 15]

Overall Significance of colistin resistance among clinical and environmental isolates:

Taken together, these findings indicate that while colistin remains one of the few last-line treatment options against MDR *A. baumannii* in India [1, 2, 7], resistance is steadily increasing in both clinical and environmental settings. The higher colistin resistance rate among clinical isolates compared to environmental isolates suggests higher selective pressure from therapeutic use of colistin [7, 10].

Plasmid mediated mcr genes are associated with transferable resistance. In the present study, the presence of both plasmid-mediated *mcr* genes and chromosomal modifications among clinical isolates [31], contrasted with the presence of a simpler single-mutation pathway among environmental isolates. Hence stringent adherence contact precautions to such patients are required [1, 2].

Overall, these results emphasise the urgent need for antimicrobial stewardship [12, 15], routine broth microdilution testing, genomic surveillance of resistance determinants, and targeted environmental decontamination to prevent further spread of colistin-resistant *A. baumannii* in healthcare settings.

Strengths and limitations:

A major strength of the present study is the combined application of the reference broth microdilution method and whole-genome sequencing to investigate colistin resistance among both clinical and patient-environmental isolates from the same healthcare setting. This integrated approach enabled direct correlation between phenotypic resistance and underlying genetic determinants. However, the

study has certain limitations. Functional validation of the identified mutations was not performed, transcriptomic analysis was not undertaken to evaluate gene expression, and the relatively small number of colistin-resistant isolates may limit the generalisability of the findings. Future studies incorporating functional genomics and larger multicentre collections would provide further insight into the evolution and dissemination of colistin resistance.

Overall, the findings of this study demonstrate that chromosomal alterations involving the *pmrAB* regulatory system, *lpxD*, and ISAba1-associated *eptA/pmrC* activation represent the predominant mechanisms of colistin resistance in MDR *A. baumannii*, whereas plasmid-mediated *mcr* genes remain relatively uncommon. The higher prevalence of resistance among clinical isolates highlights the selective pressure imposed by polymyxin use in healthcare settings. These findings emphasise the importance of routine broth microdilution susceptibility testing, genomic surveillance, antimicrobial stewardship, strict infection prevention practices, and targeted environmental decontamination to limit the emergence and spread of colistin-resistant *A. baumannii*.

Conclusion

Colistin resistance was more prevalent among clinical than environmental multidrug-resistant (MDR) *Acinetobacter baumannii* isolates. Whole-genome sequencing demonstrated that chromosomal alterations involving the *pmrAB* regulatory system, *lpxD*, and ISAba1-mediated activation of *eptA/pmrC* constituted the predominant mechanisms of resistance, whereas plasmid-mediated *mcr* genes were detected only infrequently. The absence of recognised genetic determinants in several phenotypically resistant isolates indicates that additional resistance mechanisms remain to be elucidated. These findings underscore the value of integrating reference broth microdilution with whole-genome sequencing for accurate detection and surveillance of colistin resistance. Such an approach can strengthen antimicrobial stewardship, guide infection prevention and control measures, and support strategies to limit the emergence and dissemination of colistin-resistant *A. baumannii* in healthcare settings.

Abbreviations

A. baumannii – Acinetobacter baumannii

AMRFinderPlus – Antimicrobial Resistance Finder Plus

AST – Antimicrobial Susceptibility Testing

ATCC – American Type Culture Collection

BMD – Broth Microdilution

bp – Base Pair

CLSI – Clinical and Laboratory Standards Institute

DNA – Deoxyribonucleic Acid

dsDNA – Double-Stranded Deoxyribonucleic Acid

EUCAST – European Committee on Antimicrobial Susceptibility Testing

IEC – Institutional Ethics Committee

ISAba1 – Insertion Sequence Aba1

JSSAHER – JSS Academy of Higher Education & Research

MDR – Multidrug-Resistant

MIC – Minimum Inhibitory Concentration

mcr – Mobilized Colistin Resistance

PCR – Polymerase Chain Reaction

pmr – Polymyxin Resistance

TSI – Triple Sugar Iron

WGS – Whole-Genome Sequencing

Declarations

Ethics approval and Consent:

This study was reviewed and approved by the Institutional Ethics Committee, JSS Academy of Higher Education & Research (JSSAHER), Mysuru, Karnataka, India (Approval No. JSS|MC|PG|0040|2022-2023, dated 05-04-2023). All procedures involving human participants and human-derived samples were performed in accordance with the ethical standards of the Institutional Ethics Committee, the Declaration of Helsinki and its later amendments, and relevant institutional guidelines and regulations.

The written consent to participate was obtained from all of the participants in this study **prior to sample collection.**

Consent for Publication

As the manuscript does not contain any identifiable participant information or images, the consent for publication is **"not applicable" for this study.**

Accordance statement

All procedures involving human participants and human-derived samples were performed in accordance with the ethical standards of the Institutional Ethics Committee, JSS Academy of Higher Education & Research (JSSAHER), Mysuru, India, and with the Declaration of Helsinki and its later amendments, as well as relevant institutional guidelines and regulations.

Availability of data and supplementary materials (ADM)

The data supporting the findings of this study are available within the article and its Supplementary Materials. The raw whole-genome sequencing data generated during this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number **PRJEB50614**. Supplementary Table S1 provides the isolate-wise distribution of carbapenem resistance-associated β-lactamase genes among clinical and patient-environmental multidrug-resistant *Acinetobacter baumannii* isolates

Competing Interest

"The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper."

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Authors' contribution:

Sl. No.	Author Name	Contributions
1	Monisha B	Collected the samples, performed the preliminary and main tests, Methodology, Writing - Original Draft Preparation, Visualization, Data Curation
2	Rashmi P Mahale	Conceptualization, Software, Validation, Formal Analysis, Writing - Review & Editing.
3	M Raghavendra Rao	Investigation, Resources and Project Administration.
4	Suchitra Shenoy M	Constructed the agreements and errors, Supervision.

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