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Whole-Genome Characterization of Carbapenem Resistance Determinants in Multidrug- Resistant Acinetobacter baumannii from Clinical and Patient-Environmental Isolates

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Abstract

Background

Acinetobacter baumannii is a leading cause of healthcare-associated infections and is recognized for its remarkable ability to acquire multidrug resistance. The global emergence of carbapenem-resistant *A. baumannii* (CRAB) has become a major therapeutic and public health challenge, primarily driven by the dissemination of carbapenemase-encoding genes. This study aimed to investigate the phenotypic and genomic characteristics of carbapenem resistance among multidrug-resistant (MDR) *A. baumannii* isolated from clinical specimens and corresponding patient environmental surroundings.

Methods

A prospective hospital-based study was conducted from August 2023 to October 2024 at a tertiary care hospital in Mysuru, India. A total of 110 non-duplicate MDR *A. baumannii* isolates, comprising 55 clinical and 55 environmental isolates, were included. Species identification and antimicrobial susceptibility testing were performed using standard microbiological methods and the VITEK® 2 automated system. Whole-genome sequencing was performed on all isolates to identify carbapenemase and β-lactamase genes. Genome assembly, annotation, and antimicrobial resistance gene identification were carried out using established bioinformatics pipelines, including AMRFinderPlus.

Results

Carbapenemase-associated genes were highly prevalent among both clinical and environmental isolates. The acquired carbapenemase gene *blaOXA-23* was detected in all isolates (110/110; 100%), while *blaOXA-66* and *blaNDM-1*were identified in 98 (89.1%) and 94 (85.5%) isolates, respectively. The cephalosporinase gene *blaADC-73* was detected in 41 (74.5%) clinical isolates and 35 (63.6%) environmental isolates. Additional β-lactamase genes, including *blaADC-30*, *blaTEM-1*, *blaDHA*, *blaOXA-68*, *blaADC-76*, *blaPER-7*, *blaOXA-69*, and *blaADC-191*, were detected at lower frequencies. Clinical isolates demonstrated a higher prevalence of *blaADC-73*, whereas *blaADC-30*, *blaTEM-1*, and *blaDHA* were comparatively more frequent among environmental isolates.

Conclusions

The universal presence of *blaOXA-23*, together with the high prevalence of *blaOXA-66* and *blaNDM-1*, highlights the widespread dissemination of carbapenem resistance determinants among MDR *A. baumannii* circulating in both clinical and hospital environmental settings. These findings underscore the importance of continuous genomic surveillance, robust infection prevention and control measures, and

antimicrobial stewardship programmes to limit the spread of carbapenem-resistant *A. baumannii* within healthcare facilities.

Introduction

Acinetobacter baumannii has emerged as one of the most important opportunistic pathogens responsible for healthcare-associated infections (HAIs), particularly among critically ill and immunocompromised patients. It is frequently associated with ventilator-associated pneumonia, bloodstream infections, urinary tract infections, surgical site infections, wound infections, and meningitis, contributing to prolonged hospital stays, increased healthcare costs, and high mortality rates. The remarkable ability of *A. baumannii* to survive under adverse environmental conditions, resist desiccation, form biofilms, and persist on abiotic surfaces facilitates its transmission within healthcare settings and has led to its recognition as a major nosocomial pathogen. [1, 2]

The widespread emergence of multidrug-resistant (MDR) *A. baumannii* has become a significant global public health concern. Among MDR isolates, resistance to carbapenems is particularly alarming because these agents have historically been regarded as the most effective therapeutic option for severe *A. baumannii* infections. The increasing prevalence of carbapenem-resistant *A. baumannii* (CRAB) has markedly reduced treatment options and has been associated with poor clinical outcomes, prolonged hospitalization, and increased mortality. [1–3] Recognizing the urgent need for new therapeutic strategies and improved surveillance, the World Health Organization (WHO) has classified carbapenem-resistant *A. baumannii* as a **Critical Priority Pathogen** for research and antibiotic development. [5]

Carbapenem resistance in *A. baumannii* is primarily mediated by the production of carbapenem-hydrolysing β-lactamases, although additional mechanisms, including alterations in outer membrane proteins, overexpression of efflux pumps, and changes in penicillin-binding proteins, may also contribute. [1, 4] Among the β-lactamases, class D oxacillinases are the predominant enzymes responsible for carbapenem resistance. The acquired carbapenemase gene *bla*OXA-23 is the most frequently reported determinant worldwide and has been implicated in numerous hospital outbreaks, while other acquired OXA-type carbapenemases, including *blaOXA-58*, have also been reported in several geographical regions, although with lower prevalence. Intrinsic OXA-51-like enzymes, including *bla*OXA-66, are naturally present in *A. baumannii* and may contribute to carbapenem resistance when associated with insertion sequence-mediated overexpression. [3, 4] In addition, metallo-β-lactamases such as *bla*NDM-1 confer high-level resistance to carbapenems and have disseminated rapidly across several geographic regions. Other β-lactamase genes, including *bla*ADC, *bla*TEM, *bla*DHA, and *bla*PER, further broaden the resistance spectrum and complicate antimicrobial therapy. [4, 6]

Accurate identification of carbapenem resistance determinants is essential for understanding resistance epidemiology, guiding antimicrobial therapy, and implementing effective infection prevention and control measures. Conventional phenotypic methods identify antimicrobial resistance profiles but provide limited information regarding the underlying genetic mechanisms. [7, 8] Whole-genome sequencing

(WGS) has emerged as a powerful tool for comprehensive characterization of bacterial genomes, enabling simultaneous detection of acquired antimicrobial resistance genes and chromosomal resistance determinants with high accuracy. The integration of WGS into antimicrobial resistance surveillance has substantially improved the understanding of resistance mechanisms and the dissemination of clinically important resistance genes. [3, 4, 9]

Although several studies have reported the prevalence of carbapenem-resistant *A. baumannii* in India, most have focused primarily on clinical isolates and have relied on phenotypic methods or targeted molecular assays for resistance gene detection. [7, 8] Comparative genomic studies investigating carbapenem resistance determinants among clinical isolates and corresponding patient-environmental isolates remain limited. [4, 9] Since contaminated hospital environments serve as important reservoirs for MDR *A. baumannii*, understanding the distribution of carbapenemase-associated genes in both clinical and environmental isolates is essential for strengthening infection prevention strategies and limiting hospital transmission. [2, 9]

Therefore, the present study aimed to characterize the genomic determinants of carbapenem resistance among MDR *A. baumannii* isolated from clinical specimens and corresponding patient environmental surroundings in a tertiary care hospital. Using whole-genome sequencing, the study investigated the distribution of carbapenemase-associated genes and other β-lactam resistance determinants to improve the understanding of the molecular basis of carbapenem resistance in *A. baumannii*. The findings provide valuable insights into the burden of carbapenem resistance and support the implementation of genomic surveillance and antimicrobial stewardship programmes in healthcare settings.

Materials and Methods

Study design

A prospective hospital-based study was conducted in the Department of Microbiology, JSS Medical College and Hospital, JSS Academy of Higher Education & Research, Mysuru, India, from August 2023 to October 2024. The study aimed to investigate the phenotypic and genomic characteristics of carbapenem resistance among multidrug-resistant (MDR) *Acinetobacter baumannii* isolated from clinical specimens and corresponding patient environmental surroundings.

A total of 110 non-duplicate MDR *A. baumannii* isolates were included, comprising 55 clinical isolates and 55 environmental isolates recovered from patient surroundings. Duplicate clinical isolates and repeat environmental isolates from the same patient were excluded from the study.

This study was approved by the Institutional Ethics Committee of JSS Academy of Higher Education & Research (JSSAHER), Mysuru, India (Approval No. JSS/MC/PG/0040/2022-23). All experimental protocols were approved by the Institutional Ethics Committee and were carried out in accordance with the relevant guidelines and regulations. Written informed consent was obtained from all study participants prior to sample collection.

Collection of clinical specimens

Clinical specimens, including blood, urine, sputum, endotracheal aspirates, pus, wound swabs, body fluids, and other clinically relevant samples submitted for routine culture and antimicrobial susceptibility testing, were processed according to standard microbiological procedures. [10] Blood cultures were processed using the BacT/ALERT automated blood culture system (bioMérieux, France). Positive blood cultures and all other specimens were inoculated onto MacConkey agar and 5% sheep blood agar (HiMedia Laboratories, Mumbai, India), while urine specimens were cultured on UriChrome agar (HiMedia Laboratories, Mumbai, India). Culture plates were incubated aerobically at 37°C for 18–24 h. Semi-quantitative culture was performed for urine and endotracheal aspirate specimens.

Collection of environmental specimens

Environmental sampling was performed for patients from whom MDR *A. baumannii* had been isolated from clinical specimens. Five environmental samples were collected from frequently touched surfaces surrounding each patient, including bed rails, bedside trolley, adjacent wall, floor surrounding the bed, and bathroom surfaces. Sterile cotton swabs pre-moistened with sterile normal saline were used for sample collection and transported immediately to the microbiology laboratory for processing.

Isolation and identification of Acinetobacter baumannii

Clinical and environmental samples were cultured on MacConkey agar and 5% sheep blood agar and incubated aerobically at 37°C for 18–24 h. Colonies suggestive of *Acinetobacter* spp. appeared as smooth, opaque, non-lactose-fermenting colonies on MacConkey agar and smooth, greyish-white, non-haemolytic colonies on blood agar. Urine isolates produced pale cream-coloured colonies on Uri-Chrome agar.

Presumptive isolates were identified by Gram staining and conventional biochemical tests, including catalase, oxidase, and motility testing. Gram-negative, catalase-positive, oxidase-negative, non-motile coccobacilli were considered presumptive *Acinetobacter* spp.

Clinical isolates were identified using the VITEK® 2 Compact automated identification system (bioMérieux, France), whereas environmental isolates were identified using standard biochemical tests, including indole production, urease activity, citrate utilization, and triple sugar iron (TSI) agar reactions. Whole-genome sequencing was subsequently used to confirm species identification.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of clinical isolates was performed using the VITEK® 2 Compact automated system with AST-N406 cards (bioMérieux, France). Environmental isolates were tested by the Kirby–Bauer disk diffusion method [11] on Mueller–Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines. [12] The antimicrobial panel included β-lactams, β-lactam/β-lactamase inhibitor combinations, carbapenems, aminoglycosides, fluoroquinolones, tetracyclines, folate

pathway inhibitors, and other routinely tested antimicrobial agents. Results were interpreted according to CLSI 2024 breakpoints. [12] Isolates resistant to at least one antimicrobial agent in three or more antimicrobial classes were classified as multidrug resistant.

Whole-genome sequencing

Genomic DNA was extracted from all isolates using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA concentration was determined using the Qubit dsDNA BR Assay Kit and a Qubit fluorometer (Thermo Fisher Scientific, USA). Sequencing libraries with an average insert size of approximately 450 bp were prepared using the Ultra FS II DNA Library Preparation Kit (New England Biolabs, UK). Library quality and fragment size distribution were assessed using the Agilent TapeStation system (Agilent Technologies, USA). Paired-end sequencing was performed on the Illumina MiSeq platform (Illumina, San Diego, CA, USA).

Genome assembly and annotation

Raw sequencing reads were assessed using FastQC [13] for sequence quality. Adapter trimming and quality filtering were performed before de novo genome assembly using SPAdes version 3.14 [14]. Genome assemblies were annotated using Prokka version 1.5. [15] Assembly quality was evaluated using Confindr [16], MultiQC [17], and Qualifyr, [18] and genomes meeting the predefined quality criteria were included for downstream analyses.

Identification of carbapenem resistance genes

Acquired antimicrobial resistance genes were identified from assembled genomes using AMRFinderPlus version 3.12.8 [19] with *Acinetobacter*-specific parameters. Carbapenemase-associated genes, including *bla*OXA-23, *bla*OXA-66, *bla*NDM-1, *bla*ADC-73, *bla*ADC-30, *bla*DHA, *bla*TEM-1, *bla*PER-7, *bla*OXA-68, *bla*OXA-69, *bla*ADC-76, and *bla*ADC-191, were identified. The prevalence and distribution of these genes were compared between clinical and environmental isolates.

Statistical analysis

Data were entered into Microsoft Excel and analysed using IBM SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as frequencies and percentages. Differences in the distribution of carbapenem resistance genes between clinical and environmental isolates were analysed using Pearson's chi-square test or Fisher's exact test, as appropriate. A two-tailed *P* value of < 0.05 was considered statistically significant.

The raw whole-genome sequencing reads have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject accession number **PRJEB50614.** The corresponding run accession numbers and BioSample accession numbers for all isolates are provided in **(Supplementary Table S1).**

Results

A total of 110 non-duplicate multidrug-resistant (MDR) *Acinetobacter baumannii* isolates were included in the study, comprising 55 clinical isolates recovered from patient specimens and 55 environmental isolates obtained from the corresponding patient surroundings. All isolates were confirmed as *A. baumannii* by whole-genome sequencing and were included for subsequent genomic analysis.

Phenotypic antimicrobial susceptibility

All 110 isolates exhibited a multidrug-resistant phenotype according to the Clinical and Laboratory Standards Institute (CLSI) 2024 criteria. Antimicrobial susceptibility testing demonstrated high levels of resistance to β-lactam antibiotics, including carbapenems, confirming the MDR status of all isolates included in the study. The detailed antimicrobial susceptibility profiles of clinical and environmental isolates are presented in (Table 1).

Table 1
Antimicrobial susceptibility profile of clinical and patient-environmental MDR *Acinetobacter baumannii* isolates determined using VITEK-2 system

Antimicrobial	**Clinical isolates (n = 55)**			**Environmental isolates (n = 55)**		
	S	**I**	**R**	**S**	**I**	**R**
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Colistin	0 (0.0)	55 (100.0)	0 (0.0)	13 (23.6)	42 (76.4)	0 (0.0)
Tigecycline	43 (78.2)	11 (20.0)	1 (1.8)	26 (47.3)	22 (40.0)	7 (12.7)
Minocycline	10 (18.2)	6 (10.9)	39 (70.9)	11 (20.0)	0 (0.0)	44 (80.0)
Gentamicin	6 (10.9)	2 (3.6)	47 (85.5)	6 (10.9)	4 (7.3)	45 (81.8)
Cefoperazone/ Sulbactam	4 (7.3)	0 (0.0)	51 (92.7)	8 (14.5)	4 (7.3)	43 (78.2)
Amikacin	0 (0.0)	1 (1.8)	54 (98.2)	0 (0.0)	8 (14.5)	47 (85.5)
Cefepime	0 (0.0)	3 (5.5)	52 (94.5)	0 (0.0)	9 (16.4)	46 (83.6)
Levofloxacin	1 (1.8)	1 (1.8)	53 (96.4)	0 (0.0)	8 (14.5)	47 (85.5)
Cotrimoxazole	2 (3.6)	0 (0.0)	53 (96.4)	1 (1.8)	0 (0.0)	54 (98.2)
Piperacillin/ Tazobactam	0 (0.0)	0 (0.0)	55 (100.0)	0 (0.0)	0 (0.0)	55 (100.0)
Ceftazidime	0 (0.0)	0 (0.0)	55 (100.0)	0 (0.0)	0 (0.0)	55 (100.0)
Ceftriaxone	0 (0.0)	0 (0.0)	55 (100.0)	0 (0.0)	0 (0.0)	55 (100.0)
Imipenem	0 (0.0)	0 (0.0)	55 (100.0)	0 (0.0)	0 (0.0)	55 (100.0)
Meropenem	0 (0.0)	0 (0.0)	55 (100.0)	0 (0.0)	0 (0.0)	55 (100.0)
Ciprofloxacin	0 (0.0)	0 (0.0)	55 (100.0)	0 (0.0)	0 (0.0)	55 (100.0)

Distribution of carbapenem resistance-associated genes

Whole-genome sequencing revealed a high prevalence of carbapenem resistance-associated β-lactamase genes among both clinical and environmental isolates **(**Table 2**).** The isolate-wise distribution of these genes is illustrated in **(**Fig. 1**).**

Table 2
Distribution of carbapenem resistance-associated β-lactamase genes among clinical and patient-environmental MDR *Acinetobacter baumannii* isolates

Gene	Clinical (n = 55) n (%)	Environmental (n = 55) n (%)	*P* value
blaOXA-23	55 (100.0)	55 (100.0)	NA
blaOXA-66	49 (89.1)	49 (89.1)	1.000
blaNDM-1	47 (85.5)	47 (85.5)	1.000
blaADC-73	41 (74.5)	35 (63.6)	0.216
blaADC-30	8 (14.5)	13 (23.6)	0.225
blaDHA	4 (7.3)	7 (12.7)	0.340
blaTEM-1	4 (7.3)	7 (12.7)	0.340
blaOXA-68	4 (7.3)	5 (9.1)	1.000
blaADC-76	4 (7.3)	5 (9.1)	1.000
blaPER-7	4 (7.3)	5 (9.1)	1.000
blaOXA-69	2 (3.6)	2 (3.6)	1.000
blaADC-191	2 (3.6)	2 (3.6)	1.000

Footnotes:

P values were calculated using Pearson's chi-square test or Fisher's exact test, as appropriate. Statistical significance was defined as $P < 0.05$. No statistical comparison was performed for *blaOXA-23* because the gene was detected in all the isolates in both groups.

Among the 55 clinical isolates, *blaOXA-23* was detected in all isolates (55/55; 100%). *blaOXA-66* was identified in 49 isolates (89.1%), followed by *blaNDM-1* in 47 isolates (85.5%) and *blaADC-73* in 41 isolates (74.5%). The *blaADC-30*gene was detected in eight isolates (14.5%). Lower frequencies were observed for *blaDHA*, *blaTEM-1*, *blaOXA-68*, *blaADC-76*, and *blaPER-7*, each detected in four isolates (7.3%). *blaOXA-69* and *blaADC-191* were identified in two isolates (3.6%) each.

Among the 55 environmental isolates, *blaOXA-23* was detected in all isolates (55/55; 100%). *blaOXA-66* and *blaNDM-1*were identified in 49 (89.1%) and 47 (85.5%) isolates, respectively. *blaADC-73* was detected in 35 isolates (63.6%), while *blaADC-30* was identified in 13 isolates (23.6%). *blaDHA* and *blaTEM-1* were each present in seven isolates (12.7%). *blaOXA-68*, *blaADC-76*, and *blaPER-7* were detected in five isolates (9.1%) each, whereas *blaOXA-69* and *blaADC-191*were each identified in two isolates (3.6%).

Overall, *blaOXA-23* was the predominant carbapenem resistance-associated gene, occurring in all clinical and environmental isolates. High frequencies of *blaOXA-66* and *blaNDM-1* were also observed in both

groups. Among the remaining carbapenem resistance-associated genes, *blaADC-73* was more frequently detected in clinical isolates, whereas *blaADC-30*, *blaDHA*, and *blaTEM-1* were comparatively more prevalent among environmental isolates.

Comparison of carbapenem resistance-associated genes between clinical and environmental isolates

The prevalence of the major carbapenem resistance-associated genes, *blaOXA-23*, *blaOXA-66*, and *blaNDM-1*, was identical in clinical and environmental isolates. In contrast, differences were observed in the distribution of *blaADC-73*, *blaADC-30*, *blaDHA*, and *blaTEM-1* between the two groups. The comparative distribution of carbapenem resistance-associated β-lactamase genes between clinical and environmental isolates is summarized in **(**Table 2**)**. The isolate-level distribution is shown in **(**Fig. 1**).**

The complete isolate-wise distribution of carbapenem resistance-associated β-lactamase genes is provided in Supplementary **(Table S1).**

Discussion

Carbapenem resistance in *Acinetobacter baumannii* is primarily mediated by the production of carbapenem-hydrolysing β-lactamases, particularly acquired OXA-type carbapenemases and metallo-β-lactamases, with *blaOXA-23* being the predominant resistance determinant worldwide. In recent years, the global dissemination of *blaNDM* genes has further complicated the treatment of carbapenem-resistant *A. baumannii* infections (*Chatterjee et al.*, 2016). [(20)]

In the present study, whole-genome sequencing identified *blaOXA-23*, *blaOXA-66*, *blaOXA-68*, *blaNDM-1*, *blaADC-73*, *blaADC-30*, *blaDHA*, and *blaPER-7* among both clinical and patient-environmental isolates, demonstrating the coexistence of multiple β-lactamase genes that collectively contribute to the multidrug-resistant phenotype.

The most important finding of this study was the universal detection of *blaOXA-23* in all clinical and environmental isolates, confirming its role as the predominant carbapenemase determinant in the study setting. These findings are consistent with reports by *Bae et al.* (2024) [(21)] from South Korea and *Ababneh et al*. (2025) [(22)] from Jordan, who detected *blaOXA-23* in 100% and 96.7% of clinical isolates, respectively. Similarly, *Goenka et al.* (2025) [(23)] from India and *Lee et al.* (2023) [(24)] reported prevalences of 97% and 96.6% among environmental isolates. The prevalence of *blaOXA-23* in the present study (100%) was slightly higher than that reported in most contemporary studies, reinforcing its position as the dominant carbapenemase circulating in both clinical and environmental *A. baumannii*. The widespread occurrence of *blaOXA-23* in isolates recovered from patients and their surrounding environment suggests sustained dissemination within the hospital setting, which may be facilitated by clonal spread and horizontal transfer of resistance determinants. These findings highlight the importance of strengthening infection prevention and control measures, environmental decontamination, and antimicrobial stewardship to limit the transmission of carbapenem-resistant *A. baumannii*.

The intrinsic oxacillinase gene *blaOXA-66*, a member of the *blaOXA-51*-like family, was detected in 89.1% of both clinical and environmental isolates. Comparable studies by *Ababneh et al.* (2025) [22] and *Goenka et al.* (2025) [23] reported prevalences of 72% among clinical isolates and 76% among environmental isolates, respectively. Although *blaOXA-66* is considered an intrinsic carbapenemase gene, its coexistence with acquired OXA-type carbapenemases may enhance carbapenem resistance through increased gene expression (*Chatterjee et al.*, 2016) [20]. The high prevalence observed in the present study therefore supports the widespread circulation of MDR *A. baumannii* harbouring both intrinsic and acquired carbapenem resistance determinants.

Metallo-β-lactamases constitute another important mechanism of carbapenem resistance because of their broad hydrolytic activity against β-lactam antibiotics. In the present study, *blaNDM-1* was identified in 85.5% of both clinical and environmental isolates. Similar prevalences have been reported by *Veeraraghavan et al.* (2016) [25] and *Verma et al.* (2025) [26], who detected *blaNDM-1* in 78.5% of clinical isolates and 82.0% of environmental isolates, respectively. In contrast, *Paneri et al.* (2023) [27] and *Djahmi et al.* (2020) [28] reported substantially lower frequencies of 34.0% and 42.6%, respectively. The comparatively higher prevalence observed in the present study suggests an increasing dissemination of *blaNDM-1*-harbouring *A. baumannii* within healthcare settings. The frequent coexistence of *blaNDM-1* with *blaOXA-23* is of particular clinical concern because it confers high-level resistance to carbapenems and further narrows available therapeutic options (*Chatterjee et al.*, 2016) [20].

The *blaADC-30* gene was detected in 14.5% of the clinical isolates and 23.6% of the environmental isolates. These findings are comparable with those reported by *Anggraini et al.* (2022, Indonesia) [29], who identified the *blaADC-30* variant in 12% of clinical isolates, and by *Goenka et al.* (2025, India) [23] and *Chang et al.* (2015, China) [30], who reported the *blaADC-30* variant in 16% and 12% of environmental isolates, respectively. Although ADC enzymes primarily confer resistance to cephalosporins, their coexistence with carbapenemase genes broadens the β-lactam resistance spectrum and reinforces the multidrug-resistant phenotype of *Acinetobacter baumannii* (*Chatterjee et al.*, 2016) [20]. The comparatively higher frequency of *blaADC-30* among environmental isolates may indicate the persistence and dissemination of genetically diverse resistant strains within the hospital environment.

The *blaDHA* gene was detected in 7.27% of both clinical and environmental isolates. This prevalence is comparable to that reported by *Al-Kadmy et al.* (2017, Iraq) [31], who detected *blaDHA* in 7.6% of clinical isolates, and *Chang et al.* (2015, China) [30], who reported *blaDHA* in 16.36% of environmental isolates.

Similarly, the *blaPER-7* gene was detected in 7.27% of the clinical isolates and 9.09% of the environmental isolates. Comparable frequencies have been reported by *Bae et al.* (2024, South Korea) [21], who identified *blaPER-7* in 6.1% of clinical isolates, and *Tran et al.* (2024, Vietnam) [32], who reported a prevalence of 7.27% among environmental isolates. Although *blaDHA* and *blaPER-7* were detected at relatively low frequencies, their presence alongside major carbapenemase genes may further expand the

β-lactam resistance profile and contribute to the multidrug-resistant phenotype of *Acinetobacter baumannii*, thereby limiting available therapeutic options (*Chatterjee et al.*, 2016) [20].

A low prevalence of the *blaOXA-68* gene was observed, with detection in 7.27% of the clinical isolates and 9.09% of the environmental isolates. These findings are consistent with those reported by *Ababneh et al.* (2025, Jordan) [22], who detected the *blaOXA-68* variant in 8.36% of clinical isolates and 3% of environmental isolates. Although *blaOXA-68* was identified less frequently than the major carbapenemase determinants, its coexistence with *blaOXA-23*, *blaOXA-66*, and *blaNDM-1*may contribute to the cumulative β-lactam resistance phenotype (*Chatterjee et al.*, 2016) [20]. The relatively low prevalence observed suggests that *blaOXA-68* currently plays a secondary role in carbapenem resistance within the study setting; however, continued molecular surveillance is warranted to monitor its potential dissemination.

Overall, the present study demonstrates a high burden of carbapenem resistance-associated genes among both clinical and patient-environmental *Acinetobacter baumannii* isolates. The predominance of *blaOXA-23*, together with the widespread occurrence of *blaOXA-66* and *blaNDM-1*, indicates that carbapenem resistance in the study hospital is mediated by multiple complementary resistance mechanisms. Furthermore, the detection of similar resistance determinants in clinical and environmental isolates supports the potential role of the hospital environment as a reservoir for multidrug-resistant *A. baumannii*. These findings underscore the importance of integrating genomic surveillance with routine antimicrobial susceptibility testing, reinforced infection prevention and control practices, and antimicrobial stewardship programmes to limit the dissemination of carbapenem-resistant *A. baumannii* within healthcare settings.

Limitations

This study has certain limitations. First, it was conducted at a single tertiary care hospital, which may limit the generalizability of the findings to other healthcare settings. Second, although whole-genome sequencing enabled comprehensive identification of antimicrobial resistance genes, functional validation or gene expression analyses were not performed to confirm their contribution to carbapenem resistance. Finally, the study focused primarily on β-lactam resistance determinants and did not investigate other resistance mechanisms, such as efflux pump overexpression or alterations in outer membrane proteins, which may also contribute to carbapenem resistance.

Conclusion

This study provides a comprehensive whole-genome characterization of carbapenem resistance determinants in multidrug-resistant *Acinetobacter baumannii* from both clinical and corresponding patient-environmental isolates, offering valuable insights into resistance epidemiology within a tertiary care hospital. Whole-genome sequencing demonstrated the widespread distribution of carbapenem resistance-associated genes, with *blaOXA-23* identified in all isolates, while *blaOXA-66* and *blaNDM-1*

were also highly prevalent in both clinical and environmental isolates. Additional β-lactamase genes, including *blaADC-73*, *blaADC-30*, *blaTEM-1*, *blaDHA*, *blaPER-7*, and other *blaADC* and *blaOXA* variants, further contributed to the complex β-lactam resistance profile observed among MDR *A. baumannii*.

The identification of similar carbapenem resistance determinants in clinical and environmental isolates suggests that the hospital environment may serve as an important reservoir for the persistence and dissemination of MDR *A. baumannii*. These findings underscore the value of integrating whole-genome sequencing with routine antimicrobial susceptibility testing to strengthen antimicrobial resistance surveillance and support timely infection control interventions. Continued genomic surveillance, reinforced infection prevention and control measures, and effective antimicrobial stewardship programmes are essential to limit the spread of carbapenem-resistant *A. baumannii* and preserve the effectiveness of available antimicrobial therapies.

Abbreviations

AMR: Antimicrobial resistance

AST: Antimicrobial susceptibility testing

bp: Base pairs

CLSI: Clinical and Laboratory Standards Institute

CRAB: Carbapenem-resistant Acinetobacter baumannii

DNA: Deoxyribonucleic acid

HAI: Healthcare-associated infection

IEC: Institutional Ethics Committee

JSSAHER: JSS Academy of Higher Education & Research

MDR: Multidrug-resistant

NCBI: National Center for Biotechnology Information

SRA: Sequence Read Archive

TSI: Triple Sugar Iron

WHO: World Health Organization

WGS: Whole-genome sequencing

Declarations

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Ethical committee approval: This study was approved by the Institutional Ethics Committee, JSS Medical College, JSS Academy of Higher Education & Research (JSSAHER), Mysuru, Karnataka, India (Approval No. JSS/MC/PG/0040/2022-23). The study was conducted in accordance with the ethical principles of the **Declaration of Helsinki** and the **Indian Council of Medical Research (ICMR) National Ethical Guidelines for Biomedical and Health Research Involving Human Participants.** Written informed consent was obtained from all study participants prior to sample collection.

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Informed Consent: Informed consent was obtained for experimentation and that it conforms to the standards currently applied in the country of origin.

Clinical trial registry: The authors have not registered this study with the Clinical Trial Registry as it is not applicable.

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.

Data Availability Statement: 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 materials: 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

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We understand the serious implications of image manipulation and pledge that all visual data presented in our manuscript is both authentic and adheres to the highest standards of scientific integrity.

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Figures

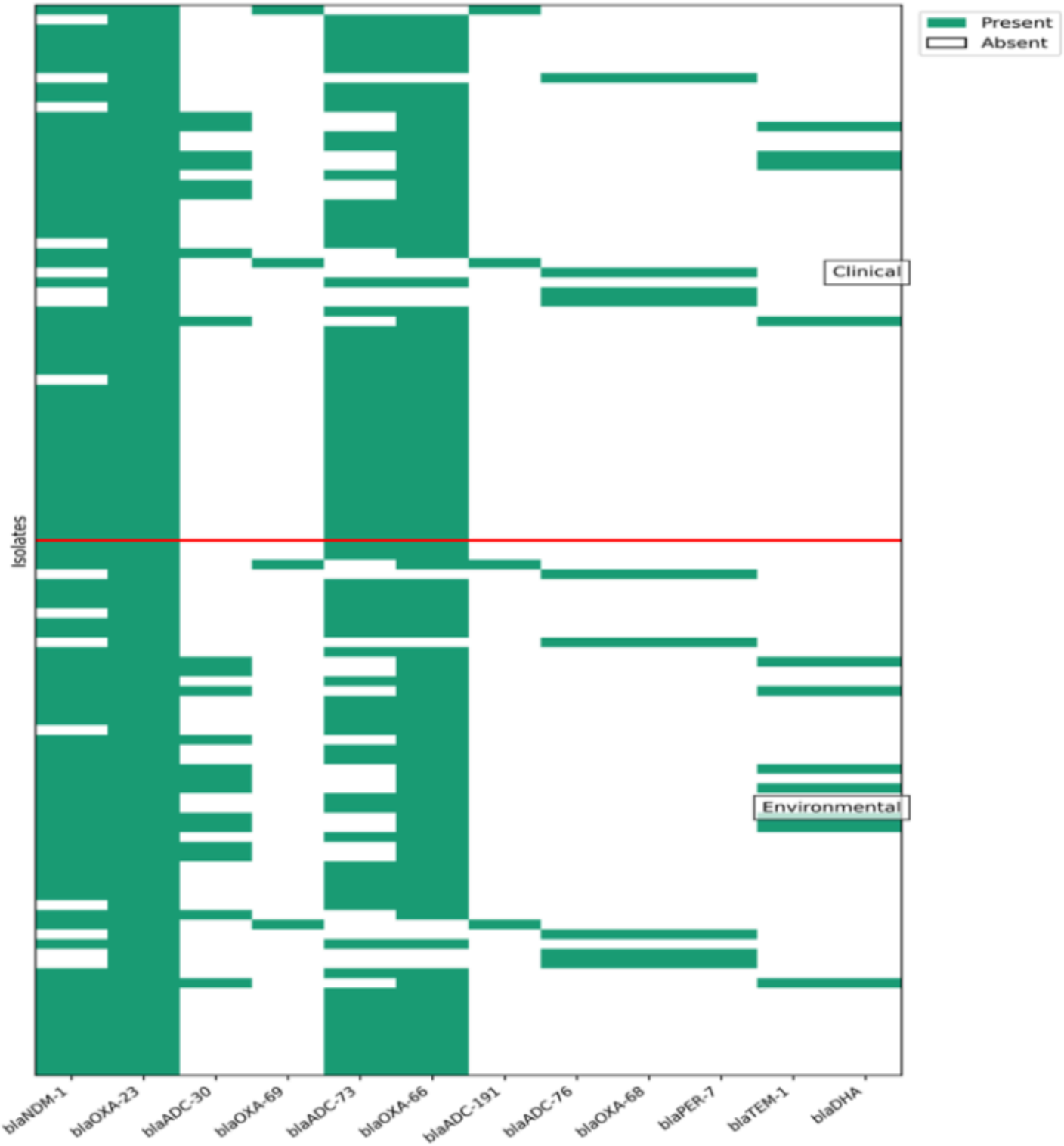


Figure 1

Heatmap showing the isolate-wise distribution of carbapenem resistance-associated β-lactamase genes among clinical and patient-environmental multidrug-resistant *Acinetobacter baumannii* isolates.

Supplementary Files

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