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Genotypic Characterisation of Carbapenemases in Clinical Isolates of *Acinetobacter baumannii* in a Tertiary Care Teaching Hospital of Kerala

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ABSTRACT

Background: The multidrug-resistant and carbapenem-resistant *Acinetobacter* baumannii strains are an increasing global concern. The primary cause of carbapenem resistance in *A. baumannii* is production of various beta-lactamases with versatile hydrolytic capacities. The present study investigates the prevalence of different carbapenemases among clinical isolates of carbapenem-resistant *A. baumannii* from a tertiary care teaching hospital in Kerala, India.

Methods: Non-duplicate isolates from sputum, endotracheal aspirate, pus, urine, and blood samples were included in the study. Isolate identification and antibiotic susceptibility testing of the isolates were done using vitek 2 system. A conventional polymerase chain reaction was used to detect the carbapenemase-encoding genes in the isolates.

Results: A total of the 126 isolates studied, among these 105 (83.3%) isolates were from intensive care unit patients and 21(16.6%) were from non-ICU inpatients. Most of the isolates were obtained from respiratory specimens-78(61.9%) followed by pus-33(26.2%), urine-12(9.5%) and blood- 3(2.4%). The prevalence of carbapenemase genes were as follows:blaOXA-51(n=126, 100%) blaOXA-23 (n=98, 77.7%), blaOXA-58 (n=7, 5.6%), blaNDM (n=66, 52.4%), blaIMP (n=26, 20.6%), and blaVIM (n=20, 15.9%). Among the total isolates 78 (61.9%) isolates harbored metallo-beta-lactamase genes, 47(37.3%) isolates harboured a single carbapenemase gene, while 69(54.8%) isolates harbored two or more genes.

Conclusion: This study reveals a higher prevalence of metallo-beta lactamases and the co-occurrence of multiple carbapenemase genes in Carbapenem resistant *A. baumannii* isolates.

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Introduction

Acinetobacter baumannii is a Gram-negative, non-fermenting bacillus that causes nosocomial infections worldwide. It causes various hospital-acquired infections, including meningitis, bacteremia, ventilator-associated pneumonia, urinary tract infections and wound infections (1). *A. baumannii* poses a significant threat due to multidrug resistance, particularly in intensive care units, where the uncontrolled use of antibiotics contributes to its emergence. Carbapenem-resistant *A. baumannii* (CRAB) isolates have emerged in hospitals globally, and their prevalence ranges from 40% to 85% across various hospitals in India (2-4). According to the World Health Organization, carbapenem-resistant *A. baumannii* is among the top-priority pathogens (5).

Production of various carbapenem hydrolyzing beta-lactamases, specifically class D beta-lactamases, has been identified as the most common mechanism of carbapenem resistance in A. baumannii. Class D beta-lactamases include the intrinsic OXA-51 and the acquired OXA-23-like, OXA-58-like, OXA-24/40-like, OXA-235-like, and OXA-143-like beta-lactamases. In addition, class B beta-lactamases, also known as metallo-beta-lactamases (MBLs), play a significant role in carbapenem resistance among *A. baumannii* isolates (6). Several studies have been performed worldwide to analyze the mechanism of carbapenem resistance in *A. baumannii*. There is paucity of such studies from India, especially in the south Indian state of Kerala.

The present study aimed to study the prevalence of various carbapenemase genes in *A. baumannii* isolates obtained from various clinical samples.

Materials and Methods

This cross-sectional descriptive study was conducted in the Department of Microbiology, Jubilee Mission Medical College and Research Institute, Thrissur, from December 2020 to May 2022. The institutional research and ethics committee approved this study (IEC Study Ref.N0:49/20/IEC/JMMC&RI).

Sample collection

The study included clinical isolates of CRAB (meropenem or imipenem MIC >8 µg/ml) obtained from sputum, endo tracheal aspirate, pus, urine, and blood samples. Samples were processed as per standard microbiology procedures.(7) Repetitive isolates from the same patients and isolates obtained in mixed cultures were excluded. All isolates were stored inside a -20 ºC deep freezer till the molecular analysis of the study.

Identification of the isolates and antibiotic susceptibility testing

The preliminary identification of the isolates were done by colonial morphology, Gram staining, motility, oxidase, glucose fermentation and nitrate reduction test. Oxidase-negative, non-lactose fermenter, non-motile, non-nitrate reducing Gram-negative coccobacilli were identified as *Acinetobacter* spp. Identification of the isolates were confirmed in the Vitek 2 compact system (Biomerieux, France)

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using Vitek 2 N281 cards following the manufacturer's instructions.

Detection of carbapenemases encoding genes

Isolates resistant to either meropenem or imipenem (MIC >8 µg/ml) were subjected to molecular characterization by conventional polymerase chain reaction(PCR). The genes, primers used and PCR amplicon size are listed in Table 1(8,9). *Escherichia coli* ATCC 25922 was used as a negative control in all PCR reactions. The

in-house NDM gene positive isolate was used as a control for PCR amplification. Two to three colonies from the fresh cultures of the isolates were suspended in 20 µl of sterile nuclease-free water and vortexed to get a uniform suspension. Direct colony PCR was done with this suspension. Briefly, PCR reactions contained 5 µl of genomic DNA, 25 µl PCR Master Mix (containing Taq DNA polymerase), 2X PCR Buffer, and 16.8 pM of each forward and reverse primer in a final volume of 30 µl. PCR amplification began with an initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95°C for 50 s, annealing at 55 °C for 40 s, and extension at 72 °C for 2 min, and a final extension step at 72 °C for 5 min. PCR products were run in 2% agarose gel and photographs were captured and analysed using Syngene XRQ gel documentation system.

DNA Sequencing

DNA sequencing (Sanger sequencing) was outsourced (Eurofins Genomics India, Bangalore) and conducted for the purified PCR products of randomly selected samples that were positive for blaOXA-51, blaOXA-23, blaOXA-58, blaNDM, blaIMP, and blaVIM genes. The generated sequences were analyzed using FINCHT TV software and BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_SPEC=GeoBlast&PAGE_TYPE=BlastSearch) to confirm the amplified PCR products.

Results

A total of 126 CRAB isolates were included in this study. Most isolates were obtained from male patients(72.2%). The patients' mean age was 50 years, the most frequent age group was 41-65 years (48.4%), followed by the elderly population > 65 years (29.4%). Among the total isolates 105(83.3%) isolates were obtained from patients admiited in Intensive Care Units(ICU) patients and 21 (16.7%) from non ICU inpatients (Table 2).

Table 1. Primer used for amplification of carbapenamase genes.

Targets	Primers	Sequences	Amplicon size (bp)
blaOXA-23	Forward Reverse	F=5′-GATCGGATTGGAGAACCAGA-3′ R=5′- ATTTCTGACCGCATTTCCAT-3′	501
blaOXA-51	Forward Reverse	F=5′-TAATGCTTTGATCGGCCTTG-3′ R=5′- TGG ATTGCACTTCATCTTGG-3′	353
blaOXA-58	Forward Reverse	F=5′-AAGTATTGGGGCTTGTGCTG-3′ R=5′- CCCCTCTGCGCTCTACATAC-3′	599
blaNDM	Forward Reverse	F=5′-GGTTTGGCGATCTGGTTTTC-3′ R=5′- CGGAATGGCTCATCACGATC-3′	627
blaIMP	Forward Reverse	F=5′-TGAGCAAGTTATCTGTATTC-3′ R=5′- TTAGTTGCTTGGTTTTGATG-3′	740
blaVIM	Forward Reverse	F=5′-ATTGGTCTATTTGACCGCGTC-3′ R=5′- TGCTACTCAACGACTGAGCG-3′	780

Table 2. Demographics of the study population.

Variable	Number (%)
Age group	
0-14	15(11.9%)
15-40	13 (10.3%)
41-65	61 (48.4%)
>65 Years	37 (29.4%)
Gender	
Male	91 (72.2%)
Female	35 (27.8%)
Location of patients	
ICUs	105(83.3%)
Ward	21(16.7%)
Comorbidities	
Diabetes Mellitus	63(50%)
Hypertension	41(32.5%)
COPD	6(4.7%)
CKD	6(4.7%)

Table 3. Antibiotic susceptibility profile of the isolates.

Antibiotics	Sensitive	Intermediate	Resistant
Piperacillin -Tazobactam	0	0	100%
Ceftazidime	0	0	100%
Cefoperazone -Sulbactam	13.5%	19%	68%
Cefepime	0	0	100%
Gentamicin	4%	16.7%	79.3%
Ciprofloxacin	0	0	100%
Imipenem	0	0	100%
Meropenem	0	0	100%
Minocycline	50%	0.8%	49.2%
Trimethoprim-Sulfamethoxazole	22.2%	0	77.8%
Tigecycline	57.1%	38.1%	4.8%
Colistin	0.00%	100%	0.00%

Table 4. CRAB isolates harboring single gene (N=47).

Genes	Number of isolates
bla OXA 23	38
bla IMP	2
Bla NDM	7

Table 5. CRAB isolates harboring more than one gene(N=69).

Carbapenemase genes	Number of isolates
OXA 23 & NDM	28
OXA 23, NDM & IMP	14
OXA 23, NDM & VIM	8
OXA 23 & IMP	5
OXA 23 & VIM	3
OXA 58 & NDM	3
OXA 23, IMP & VIM	2
OXA 23, NDM, IMP& VIM	2
OXA 58, NDM & IMP	2
NDM, IMP & VIM	1

The isolates included in this study were obtained predominantly from respiratory specimens-78(61.9%) followed by pus -33(26.2%), urine - 12(9.5%) and blood-3(2.4%). Among the 126 isolates, 72 (57.1%) isolates were susceptible to tigecycline, 63 (50%) to minocycline, and 28 (22.2%) to trimethoprim-sulfamethoxazole. All the isolates showed intermediate susceptibility to colistin (Table 3).

PCR detection of carbapenemase genes

All the 126 isolates studied were positive for the intrinsic blaOXA-51 gene and 116 (92%) isolates were positive for any of the acquired carbapenemase encoding genes. Most common acquired carbapenemase gene detected was blaOXA-23 (77.7%) followed by blaNDM gene in (52.4%) (Fig.1); Additionally, 47(37.3%) isolates harbored single gene whereas 69(54.8%) isolates harbored two or more genes (Table 4 and 5). Metallo-beta-lactamases were detected in 78 out of 126 (61.9%) isolates. The amplified products were confirmed and matched 100% using NCBI and the BLAST tool. (The blast details are given as supplementary Data).

Discussion

Carbapenem-resistant *A. baumannii* has emerged as a threat to patients admitted to ICUs worldwide, including in the Indian subcontinent.

Production of various carbapenemases is the primary cause of carbapenem resistance in these multidrug-resistant organisms. Studies to understand the genetic mechanisms of carbapenem resistance can facilitate the discovery of new drugs for the treatment of these difficult-to-treat infections. The present study was conducted in an 1800-bed tertiary care teaching hospital catering to patients from three neighboring districts of central Kerala.

Similar to previous studies, all isolates in our study were confirmed to harbor the OXA-51 gene, an intrinsic carbapenemase gene in *A. baumannii*. However, it produces a weak carbapenemase. Among the isolates, 92% were positive for one or more acquired carbapenemase-encoding genes. In concordance with the findings of the previous studies in India, blaOXA-23 (77.7%) was the predominant gene detected in this study (10-13). Kalal et al. (2020) and Vijayakumar et al. have reported a higher prevalence of OXA23 (91% and 100%, respectively) (14,15).

In the present study, the blaOXA-58 gene was detected in only 5.6 % of the isolates. Similar to our results, other studies in Poland, China, and Egypt detected a lower prevalence of the blaOXA-58 gene among carbapenem-resistant *A. baumannii* isolates (16-18). A higher prevalence of blaOXA-58-like genes has been reported in Pakistan and Middle Eastern countries (19-21). Compared to previous studies in India, the prevalence of the blaOXA-58 gene shows a rising trend (22,23). Therefore, more studies are needed from different regions in India before we can conclude that the prevalence of blaOXA-58 is low among carbapenem-resistant *A. baumannii*

In the present study, nearly two-thirds of the isolates were positive for genes encoding MBLs, and blaNDM was the prevalent one. Compared to previous studies in India, our results showed an increased prevalence of MBLs among CRAB isolates.(23,24) In a study in New Delhi, no NDM gene was detected among the 100 isolates studied (23). B.S Kalal et al. reported the blaNDM, blaIMP, and blaVIM genes in 13%, 16%, and 64% of the isolates (14). This shows an increasing prevalence of MBLs among CRAB isolates and a difference in the distribution of MBL genes in different geographical areas of the country. The coexistence of blaNDM and other carbapenemase coding genes demonstrates the isolates' capacity to obtain new genes despite having the blaOXA carbapenemase gene. The rising occurrence of metallo-beta-lactamases is concerning as it poses a potential risk of resistance to all effective betalactam antibiotics available for treatment of infections caused by *Acinetobacter baumannii*.

Our results show that different combinations of oxacillinases and Metallo-beta-lactamases are prevalent among *A. baumanni* clinical isolates. The presence of multiple combinations of resistant genes such as blaOXA-23, blaOXA-58, blaNDM, blaIMP, and blaVIM in the isolates also shows considerable genetic heterogeneity among the clinical isolates from the same center. Multiple hospital admissions and visits to different institutions may be the reason for this genetic diversity.

Our study had some limitations. In this study, we have analyzed only the carbapenemase genes previously reported in India. Hence, the presence of rare genotypes in our isolates can not be ruled out. In our study, except for the carbapenemase gene, other mechanisms of carbapenem resistance have not been investigated.

Conclusion

In conclusion, there has been a significant increase in the prevalence of Metallo-beta-lactamases and co-occurring multiple carbapenemases among clinical isolates of *A. baumannii*. This is a cause for serious concern as this further exacerbates the already limited treatment options for CRAB infections, highlighting the urgent need for new and innovative treatment strategies. The problem of antibiotic resistance is a global health threat that

requires immediate and concerted action to prevent it from spiraling out of control.

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Ethics approval and consent to participate

Not needed.

Conflict of interest

The authors declare no conflicts of interest associated with this manuscript.

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