Journal of Infectious Diseases and Therapy
ISSN: 2332-0877

Seshatri et al., J Infect Dis Ther 2022, 10:7

Research Article Open Access

Frequency of OXA-Type Carbapenemases among Carbapenem Resistant *Acinetobacter baumannii* in Clinical Isolates from Adult Intensive Care Unit in India

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Abstract

Purpose: *Acinetobacter baumannii* is a highly virulent bacterium in modern healthcare, with a high ability to acquire antimicrobial resistance. Carbapenemases production appears to be the most common mechanism involved in drug resistance to carbapenem. As the prevalence of carbapenem resistant *Acinetobacter baumannii* was high in ICU patients, this study was designed to find the frequency of OXA genes including OXA 23, OXA 24, OXA 51, and OXA 58.

Methods: A clinical specimen was collected from patients admitted to the adult intensive care unit. DNA was isolates from Carbapenem resistant *Acinetobacter baumannii* and amplified using conventional PCR technique and gel electrophoresis for visualization of results.

Results: The frequency of the OXA 23 gene was high with 87.5%, followed by OXA 51 gene with 73.2%. All 56 isolates were negative for the OXA 24 and OXA 58 genes. We also found that both OXA 23 and OXA 51 genes co-existed in 40 (71.4%) isolates. No significant difference was found between drug resistance genes (OXA 23 and OXA 51) and clinical outcomes. The relationship between the presences of OXA gene was compared between survivors and non-survivors, which was found out to be non-significant. The presence of OXA genes showed no significant increase in the length of hospital stay. Association of APACHE IV scores with clinical outcome were calculated, and was found out to be significant in discharge *vs.* expired group.

Conclusion: Early detection of these drug resistant genes by molecular methods is essential in decreasing the spread of carbapenem resistant *Acinetobacter baumannii*.

Keywords: Acinetobacter; Antimicrobial resistance; OXA enzymes; Carbapenemases; Molecular diagnosis

Introduction

Acinetobacter baumannii is the significant pathogen causing infection widely in hospitals. In most acinetobacter species infections, such as ventilator associated pneumonia, meningitis, bacteremia, peritonitis, urinary tract infections, and wound infections, *Acinetobacter baumannii* accounts for about 90%. Multidrug resistant Acinetobacter baumannii infection mostly occurs in critically ill patients admitted to an Intensive Care Unit (ICU) and it is associated with a high mortality rate, ranging from 26% to 68% [1].

The accumulation of different resistance mechanisms has gradually lessened the use of number of antimicrobial agents available to treat *Acinetobacter baumannii* infections in clinical practice. The known resistance mechanisms include enzymatic degradation of drugs by β-lactamases and aminoglycoside modifying enzymes, permeability defects, multidrug efflux pumps, and modification of target sites [2].

Production of carbapenemases either by acquired or naturally occurring Oxacillinases (OXA) genes appears to be the most familiar mechanism seen with carbapenem resistant *Acinetobacter baumannii*. The carbapenemase enzymes are mediated by the ambler class D β-lactamases and ambler class B Metallo-β-Lactamases (MBL), which is of greater concern. Since 2000, the gradual development of carbapenem resistance is seen predominantly due to the emergence of the Ambler Class D Oxacillinases. The five subtypes of OXA genes are OXA 23-like (OXA 23, OXA 27, OXA 49, and OXA 239); OXA 24-like (OXA 24, OXA 25, OXA 26, OXA 40 and OXA 72); OXA 51-like (which is intrinsic to Acinetobacter baumannii), OXA 58 and OXA 143-like (OXA 143 and OXA 231). The OXA 51-like group establishes a chromosomal enzyme family naturally present in Acinetobacter baumannii. Carbapenem resistance in Acinetobacter baumannii is mediated more frequently by OXAs and less frequently by MBL [3].

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Received: 04-August-2022, Manuscript No. jidt-22-71248; **Editor assigned:** 09-August-2022, PreQC No. jidt-22-71248; **Reviewed:** 23-August-2022, QC No. jidt-22-71248; **Revised:** 30-November-2022, Manuscript No. jidt-22-71248; **Published:** 28-December-2022, DOI: 10.4172/2332-0877.1000520

Citation: Seshatri S, Charan J, Tak V, Nag VL, Ambwani S, et al. (2022) Frequency of OXA-Type Carbapenemases among Carbapenem Resistant Acinetobacter baumannii in Clinical Isolates from Adult Intensive Care Unit in India. J Infect Dis. 10: 520.

Objective: To determine the frequency of OXA-type carbapenemases among carbapenem-resistant *Acinetobacter baumannii* in clinical isolates from adult ICU and to evaluate the relationship between OXA gene resistance and clinical outcome.

Materials and Methods

Study design

Settings: After getting approval from Institutional Ethics Committee (AIIMS/IEC/2019-20/847), the clinical specimen of the patients admitted in adult ICU was collected and sent to the department of microbiology for culture and sensitivity from June 2019 to March 2021.

Participants: Patients with carbapenem resistant *Acinetobacter baumannii* infection in adult ICU and who gave written informed consent were included in the study.

Variables: The isolates were obtained from various clinical specimens such as blood samples, urine samples, Bronchoalveolar Lavage (BAL), and wound samples [4].

Data sources/measurements: A total of 56 patients were included in the study and their demographic details were recorded in case record form. A brief clinical history was recorded including the patient's chief complaints, comorbidities like diabetic mellitus, hypertension, coronary heart disease, thyroid disorder, and history of any surgery. General physical examination of the patients was done, which included anaemia, cyanosis, and jaundice. APACHE IV score was calculated for all the patients who are enrolled in the study. Bacterial identification was performed by routine conventional gram staining, microbial culture, and biochemical tests using the standard recommended techniques. Antimicrobial susceptibility testing was done using E (Epsilometer) Strip test to determine the Minimum Inhibitory Concentration (MIC) of meropenem and imipenem. MIC values of ≥ 8 µg/ml were taken as resistant for both meropenem and imipenem according to CLSI 2019 guidelines [5].

Genomic DNA was extracted from bacterial isolate by using DNA extraction kits. DNA concentrations were determined by the nanodrop technique at 260 nm. Amplification of target genes OXA 23, OXA 24, OXA 51 and OXA 58 was done by conventional PCR. Primer pair was used to amplify OXA genes are OXA-23 (501 bp: 5-GAT CGG ATT GGA GAA CCA GA and 5-ATT TCT GAC CGC ATT TCC AT), OXA-24 (246 bp: 5-GGT TAG TTG GCC CCC TTA AA and 5-AGT TGA GCG AAA AGG GGA TT), OXA-51 (353 bp: 5-TAA TGC TTT GAT CGG CCT TG and 5-TGG ATT GCA CTT CAT CTT GG) and OXA-58 (599 bp: 5-AAG TAT TGG GGC TTG TGC TG and 5-CCC CTC TGC GCT CTA CAT AC). The amplification conditions were, initial denaturation at 94°C for 5 min 35 cycles of 94°C for 25 s, 52°C for 40 s and 72°C for 50 s, and a final elongation at 72°C for 6 min. Amplified desired DNA Fragments were verified by Agarose gel electrophoresis. Electrophoresis was carried out using bio-rad mini-sub electrophoresis system. UV transilluminator gel documentation system was used for the visualization of bands.

APACHE IV score was calculated using the online calculator, which predicts the estimated mortality rate and estimated length of ICU stay in the hospital. The variables collected within the first 24 hours after admission of study participants in an ICU were determined. APACHE IV score was calculated based on the worst values for each variable [6].

Study size: Sample size was calculated based on the previously conducted study by Vijayakumar, et al. The OXA 23 proportion in the study was 98%. Considering this as expected frequency, in this study with 99% confidence interval and 5% absolute error, the sample size was fifty-three isolates.

Statistical methods: Data were entered into Microsoft excel and was analyzed using SPSS version 23. Data were reported as mean ± Standard Deviation (SD) for continuous variables and percentage for categorical variables. Descriptive statistics were used to summarize demographic characteristics and APACHE IV score. Association between the OXA gene resistance with clinical outcome and OXA gene resistance with survivors were assessed using chi-square test. Comparisons of quantitative variables (ICU stay, ward stay, and total stay) between the two independent groups (OXA gene resistance and length of stay in the hospital) were analyzed using student "t" test. APACHE IV score was compared between three independent groups (discharge, expired, and LAMA) by using one-way ANOVA test. A p-value of <0.05 was considered statistically significant [7].

Results

In the present study, 56 patients with carbapenem resistant *Acinetobacter baumannii* infection were recruited from the adult ICU, AIIMS, and Jodhpur.

Descriptive data: Demographic characteristics, clinical examination, duration of ICU and hospital stay, and type of clinical specimens including BAL, blood, and urine and wound sample were recorded for all the patients as per the case record form submitted. The frequency of demographic and clinical characteristics is presented in Table 1.

Variables		Number (%)
Gender	Male	38 (67.9)
	Female	18 (32.1)
Age (years) (means ± SD)	-	46.36 ± 17.404
Clinical specimen	Bronchoalveolar lavage	42 (75)
	Blood	13 (23.2)
	Urine	0
	Wound sample	1 (1.8)
Reason for hospitalization	Clinical complication	15 (26.8)
	Neurological complication	15 (26.8)

	Respiratory complication	10 (17.8)
	Surgical complication	9 (16.1)
	Trauma	6 (10.7)
	Malignancy	1 (1.8)
Comorbidities	Diabetes	12 (21.4)
	Hypertension	10 (17.9)
	Chronic kidney disease	6 (10.7)
	Tuberculosis	5 (8.9)
	Thyroid disorder	2 (3.6)
	Bronchial asthma	2 (3.6)
	History of surgery	2 (3.6)

Table 1: Demographic and clinical characteristics related to patients with infections to Carbapenem-resistant *Acinetobacter baumannii* in adult ICU (n=56).

With respect to the presence of drug resistance gene among the collected isolates, OXA 23 gene was detected in 49 (87.5%) and OXA 51 gene was detected in 41 (73.2%) isolates. All 56 isolates were negative for OXA 24 and OXA 58 genes. Both OXA 23 and OXA 51 genes were co-existed in 40 (71.4%) isolates. Either OXA 23 or OXA 51 gene was present in 10 isolates, in which 9 isolates had OXA 23 gene and only one isolate had OXA 51 gene, detection of genes encoding OXA 23 and OXA 51 shown in Figure 1.

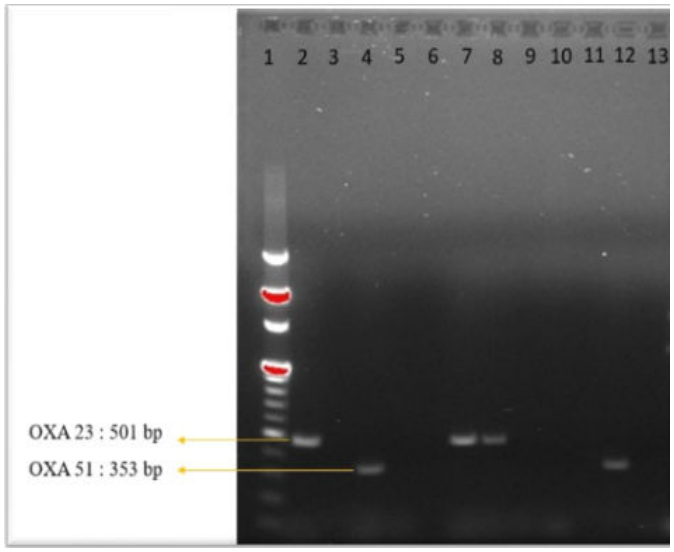

Figure 1: Detection of genes encoding OXA 23 and OXA 51. Lane 1 shows 100 bp DNA ladder, Lane 2 shows OXA 23 (Positive control), Lane 4 shows OXA 51 (Positive control), Lane 7,8 shows OXA 23, Lane 11 shows OXA 51.

The relation between OXA gene resistance (OXA 23 and OXA 51) and clinical outcomes (discharge, expired, and LAMA) was also studied. The association between clinical outcomes and OXA 23 gene, the p-value was found non-significant (p-value=0.573). For the relationship between OXA 51 and clinical outcomes, found non-significant (p-value=0.913). The presence of both the genes (OXA 23 and OXA 51) was also not associated significantly with any of the outcomes (p-value=0.905) [8,9].

The relationship between presence of OXA gene was compared between survivors and non survivors. Considering LAMA to be best case scenario, it was included in survivors. The presence of OXA 23 gene was found to be non-significant between 23 (46.9%) survivors and 26 (53.06%) non survivors with p-value of 0.443. For the relationship between OXA 51 between 19 (46.34%) survivors and 22 (53.65%) non survivors, the p-value was 0.767. Presence of both the genes (OXA 23 and OXA 51) was also not associated significantly between 19 (47.5%) survivors and 21 (52.5%) non survivors with p-value of 0.767 [10].

The severity of patient infection was evaluated by using the APACHE IV score, which was retrieved from medical charts within 24 hours of ICU admission. The mean and standard deviation of APACHE IV score was 58.66 ± 23.48 [11]. Association of APACHE IV score with clinical outcomes including discharge, expired and LAMA was calculated. The mean ± SD for discharge, expired and LAMA were 45.71 ± 14.54, 66.45 ± 26.011 and 66.25 ± 7.500 respectively with a p-value of 0.004, which was significant. After post-hoc analysis, significant value was seen between discharges *vs.* expired group (Table 2).

Outcome	APACHE IV score (Mean ± SD)	95% CI		p-value
		Lower limit	Upper limit	
Discharge	45.71 ± 14.54	39.1	52.33	0.004*
Expired	66.45 ± 26.011	56.91	75.99	
LAMA	66.25 ± 7.500	54.32	78.18	
Note: *p<0.05 is taken as significant				

Table 2: Association between APACHE IV score and Clinical outcome.

Acinetobacter baumannii is one of highly virulent bacteria in modern healthcare, with a high ability to acquire antimicrobial resistance. In clinical practice, the number of antibiotic classes available to treat Acinetobacter baumannii infection is decreasing due to the accumulation of resistance mechanisms.

Clinically carbapenems are most commonly used to treat critically ill patients with gram-negative infection resistant to conventional antibiotics. Carbapenemases production appears to be the most common mechanism involved in drug resistance to Carbapenem in *Acinetobacter baumannii* and is most commonly mediated by OXA type β lactamases and MBL [12].

The present study was done to find the frequency of OXA genes including OXA 23, OXA 24, OXA 51, and OXA 58 in carbapenem resistant *Acinetobacter baumannii* isolates from adult ICU. Out of 56 carbapenem resistant *Acinetobacter baumannii* isolates, the OXA 23 gene was present in 49 (87.5%) isolates and the OXA 51 gene was present in 41 (73.2%) isolates. OXA 23 gene was solely present in nine isolates, and the OXA 51 gene was solely present in one isolate. The carbapenemases encoded by these genes have been described to confer reduced susceptibility to carbapenems [13]. The high frequency of the OXA 23 gene was observed among different studies. The frequency of the OXA 23 gene was 58% in a hospital in Northern Taiwan. The frequency of the OXA 23 gene was 95% in Thailand. The frequency of the OXA 23 gene among carbapenem-resistant *Acinetobacter baumannii* isolates is gradually increasing (Table 3).

Outcome	Mean difference	Standard error	p-value
Discharge *vs.* expired	20.737	6.1	0.004*
Expired *vs.* LAMA	0.202	11.968	1
LAMA *vs.* discharge	20.536	11.776	0.261
Note: *p<0.05 is taken as significant			

Table 3: Post-hoc analysis of the association between APACHE IV and clinical outcomes.

The molecular characterization of carbapenem resistant *Acinetobacter baumannii* from a tertiary care hospital in the Southern part of India and exhibited the presence of the OXA 23 gene and OXA 51 gene. In contrast to our study, they found that the OXA 51 gene was highly prevalent in all isolates followed by the OXA 23 gene with 98%. A study conducted by Niranjan D, et al., in North India showed the presence of OXA 51 gene in all isolates (100%) with high frequency of OXA 23 gene (46.6%). The presence of the OXA 51 gene in all isolates (100%) and a higher frequency of the OXA 23 gene with 72.7% [14].

All isolates in our study were negative for the OXA 24 and OXA 58 genes. According to Petrova AP, findings, 97.7% of the OXA 23 gene was present and OXA 51, OXA 24 and OXA 58 genes were not present in clinical isolates of carbapenem resistant Acinetobacter baumannii from ICU patients. Similar findings were seen with Stoeva T, et al., where OXA 23 gene was present in all isolates and negative for OXA 51, OXA 24, and OXA 58 genes [15].

The high prevalence of multidrug resistance including carbapenems among Acinetobacter baumannii strains isolated from ICU patients. It showed the presence of OXA 51 in all isolates with a high prevalence of the OXA 23 gene (55.1%) followed by the OXA 58 genes with 3.6%. A study in Uruguay conducted by Bado I, et al., showed the presence of OXA 51 gene in all isolates with a high frequency of OXA 23 gene (79.5%) and OXA 58 genes with 3.8%. Wang TH, et al., failed to identify the presence of the OXA 58 gene among Carbapenem-resistant *Acinetobacter baumannii* exclusively in the blood sample in Taiwan [16]. In this study, all isolates were positive for the OXA 51 like gene with a high frequency of the OXA 23-like gene (88.1%) and 4.09% of the OXA 24 like gene.

In the present study, 40 (71.4%) isolates reported the co-existence of the OXA 23 and OXA 51 genes. A high prevalence of the OXA 51 gene followed by the OXA 23 gene with 84% co-existence. Furthermore, a study by also showed 33.3% co-existence of the OXA 23 gene and the OXA 51 gene. This indicates that the co-existence of the OXA 23 and OXA 51 genes is most common among Carbapenem resistant *Acinetobacter baumannii*. In contrast to this, Higgins PG, et al., who conducted a global study, most of the isolates had the OXA 23 and OXA 58 genes instead of the OXA 51. This suggested the clonal spread of a resistant organism, but it was not associated with a particular cluster.

A few studies showed the presence of all four OXA genes that is OXA 23, OXA 24, OXA 51, and OXA 58 genes. Khajuria A, et al., studied the prevalence of OXA and MBL genes in carbapenem resistant *Acinetobacter baumannii* among the Indian population in Central India. It showed a high frequency of the OXA 23 gene with 52.4%, followed by the OXA 51, OXA 58, and OXA 24 genes with 44.8%, 14.3%, and 9.52% respectively. A similar report was published by Amudhan S, et al., in the South Indian population, where OXA 51 gene was present in 93.4%, followed by OXA 23, OXA 24, and OXA 58 in 89.6%, 1.8%, and 0.94% respectively. Simo Tchuinte, PL et al., study in Madagascar showed OXA 51 like gene was present in all isolates. OXA-23, OXA-24, and OXA-58 genes were present in 53.3%, 13.3% and 6.7% of carbapenem-resistant *Acinetobacter baumannii* isolates, respectively.

Carbapenem resistance due to the synthesis of OXA-type carbapenemases is growing drastically. OXA enzymes are the most important reason for resistance to Imipenem and Meropenem in *Acinetobacter baumannii* infection worldwide. All isolates in our study were resistant to Imipenem and Meropenem with MIC values above 32 µg/ml. A study in Madagascar, where all the isolates showed MIC value similar to that of our study [17].

The synthesis of β-lactamase enzyme, that is oxacillinases, might be a molecular mechanism of carbapenem resistance among the *Acinetobacter baumannii* isolates evaluated in our study. The recent emergence and dissemination of the OXA 23 gene have been reported in India and in other countries such as Poland, Brazil, Italy, Spain, and Portugal represent a major mechanism of resistance to imipenem and meropenem among clinical isolates of *Acinetobacter baumannii*. The insertion element ISAba1 is the most important factor associated with the increased expression of OXA genes. Especially, upstream of the OXA 23 and OXA 51 genes by ISAba1 has been shown to be associated with Carbapenem resistance in *Acinetobacter baumannii* isolates [18].

Bronchoalveolar lavage was the clinical specimen most commonly associated with the carbapenem resistant *Acinetobacter baumannii*. This indicates that the use of mechanical ventilation as clinical support

is contributing to the spread of infections. Dias VC, et al., also isolated carbapenem resistant *Acinetobacter baumannii* more frequently in the tracheal aspirate [19].

We also studied the relationship between the presence of the OXA gene resistance and clinical outcomes including discharge, expired, and LAMA. There was no significant difference was found between OXA 23 gene and OXA 51 gene with clinical outcomes, and the p-value was 0.573, and 0.913 respectively. The presence of both the genes (OXA 23 and OXA 51) was also not associated significantly with any of the clinical outcomes (p-value=0.905) [20].

Discussion

In our study, the APACHE IV score, the most recent version of the APACHE scoring system was used for the prediction of estimated mortality rate and estimation of the length of ICU stay in the hospital. The mean and standard deviation of APACHE IV score was 58.66 ± 23.48. The mean predicted length of ICU stay by the APACHE IV model was 6.44 ± 1.76 and the estimated length of the mortality rate was 22.59 ± 18.25. Association between the APACHE IV score and clinical outcome including discharge expired and LAMA was calculated and found to be significant with the p-value of 0.004. In the post-hoc analysis, the p-value was significant in the discharge *vs.* expired group. Bado I, et al., used the APACHE II scoring system for the estimation of the length of ICU stay in the hospital. The mean predicted length of ICU stay by APACHE IV model was 23.1 ± 6.0 and the estimated mortality rate was 14.7 ± 12.1. APACHE II scoring system was developed in 1985 as a modification of the original APACHE score. This study did not compare the molecular characterization of carbapenem resistant *Acinetobacter baumannii* with the clinical outcomes of the patients admitted to the ICU.

Multidrug resistant *Acinetobacter baumannii* infection mostly occurs in critically ill patients in ICU and it is associated with high mortality, ranging from 26% to 68%. So, we also studied and compared the OXA gene resistance with survivors and non-survivors. The presence of the OXA 23 gene was found to be non-significant between survivors and non-survivors with a p-value of 0.443. The OXA 51 gene was found to be non-significant between survivors and non-survivors with a p-value of 0.767. The presence of both the genes (OXA 23 and OXA 51) was found to be non-significant between survivors and non-survivors with a p-value of 0.767.

The relationship between OXA gene resistance and length of stay in the hospital including ICU stay, ward stay, and total stay was compared. The presence of OXA 23 gene showed an increase in ICU stay and total stay with a p-value of 0.098 and 0.797 respectively. The presence of OXA 51 gene showed an increase in ICU stay, ward stay, and total stay with a p-value of 0.094, 0.859 and 0.206 respectively. The presence of both OXA 23 and OXA 51 gene showed an increase in ICU stay and total stay with a p-value of 0.252 and 0.642 respectively. However, we did not find any significant difference between the presence and absence of the OXA 23, OXA 51, and both OXA 23 and OXA 51 genes with the length of hospital stay.

Conclusion

Our study, reports the emergence of the OXA 23 and OXA 51 genes as the predominant cause of carbapenemases production among clinical isolates of Carbapenem-resistant *Acinetobacter baumannii* infections in our health care settings. It also has major significance for both antibacterial therapy and prognosis of infectious disease, and infection control. Hence, early detection of these drug-resistant genes by molecular methods is essential in decreasing the spread of carbapenem-resistant *Acinetobacter baumannii*.

The strengths of our study are that we compared the relationship between OXA gene resistance with a length of stay in the hospital as well as clinical outcomes including discharge, expired, and LAMA. We used the most recent version of the APACHE scoring system, the APACHE IV score for analyses of an association with clinical outcomes.

Limitation

We studied only the frequency of OXA-type carbapenemases producing genes, but co-existence with Metallo-β-lactamases producing genes was not studied.

Acknowledgement

We thank staff members and technicians of department of pharmacology and microbiology for technical support.

Funding

This research was funded by AIIMS, Jodhpur.

Ethical Approval and Consent Participation

Yes, approved from Institutional Ethics Committee, AIIMS, Jodhpur (AIIMS/IEC/2019-20/847).

Conflict of Interest

None.

Ethical Issues

None.

Author's Contribution

SA, VLN, VT and SS conceived the study and reviewed the methodology. JC did data analysis. All authors did literature review, drafted the manuscript, reviewed the work critically, and approved the final draft of the manuscript for submission.

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