Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

"TO STUDY THE MOLECULAR CHARACTERIZATION OF INVASIVE CARBAPENEM-RESISTANT *ACINETOBACTER BAUMANNII* WITH SPECIAL REFERENCE TO BLAOXA-23 GENE IN ICU PATIENTS

Dr. Snehanshu Shukla, Dr. Gaurav Kumar, Dr. Rajesh Verma, Dr. Nashra Afaq, Dr. Deepika Shukla, Dr. Vikas Mishra[**]

Professor[1], Department of Microbiology, Rajarshi Dashrath Autonomous State Medical College,Ayodhya, India.
Associate Professor[2], Department of Microbiology, Rajarshi Dashrath Autonomous State MedicalCollege, Ayodhya, India.
Professor and Head[3], Department of Microbiology, UPUMS Saifai Medical College, Etawah,India.
Research Associate[4], Department of Microbiology, Rama Medical College Hospital andResearch Centre, Kanpur, Uttar Pradesh, India.
Assistant Professor and Head[5], Department of Microbiology, Maharana Pratap Dental Collegeand Hospital Kothi, Kanpur, Uttar Pradesh, India.

Professor[**], Department of Microbiology, G.S.V.M. Medical College, Kanpur, Uttar Pradesh,India
Corresponding author: Dr. Vikas Mishra[**]

Email: drmishravikas@gmail.com

ABSTRACT

INTRODUCTION: *A. baumannii* strains are proven to be highly resistant to the majority of currently used antimicrobial drugs, including carbapenems. In this species, carbapenem resistance is mostly caused by β-lactamase. Carbapenem-hydrolyzing class D oxacillinases are common among multidrug-resistant (MDR) *A. baumannii* strains. The current investigation was done to assess the presence and distribution of blaOXA genes among multidrug-resistant *A. baumannii* isolated from the ICU.

AIM AND OBJECTIVES: To study the Molecular Characterization of Invasive Carbapenem-Resistant *Acinetobacter baumannii* with special reference to blaOXA-23 gene in ICU patients at a Tertiary care centre.

MATERIAL AND METHODS: This was a Cross sectional study carried out in the Department of Microbiology for a period of 1 year i.e, March 2023 to March 2024 at a tertiary care centre. A total of 130 non-duplicate, consecutive, carbapenem-resistant isolates recovered from *Acinetobacter* species were included in this study. The isolates were obtained from the clinical samples. The isolates were identified by the standard biochemical tests and the Antimicrobial

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024
susceptibility testing was performed according to the CLSI guidelines 2023. The DNA was extracted using the Qiagen DNA extraction kit as per the manufactures guidelines and the gene blaOXA was detected using the conventional PCR assay.

RESULTS: In the present study a total of 1216 clinical samples were collected in which 130 Acinetobacter species were isolated. The maximum number of isolates were from the ETA samples with 98 (75%), 25 (19.2%) from blood and 7 (5.3%) from the tissue. The ratio of Males 78 (60%) was more as compared to that of the Females 52 (40%) with the maximum age of 31-40 being affected the most followed by 41-50 and least in the age group above 61 years of age. Antimicrobial susceptibility testing revealed that all the isolates were resistant to ceftazidime, cefepime, piperacillin/tazobactam, cefoperazone/sulbactam, aztreonam, imipenem, meropenem, amikacin, netilmycin, tetracycline, tobramycin, levofloxacin and co-trimoxazole. In the present study it was also observed that around 126 (96.9%) of the study isolates were susceptible to polymyxin B (colistin).

The Molecular characterization reveals that among the 130 isolates tested blaOXA-23 gene was present in all the 130 isolates (100%).

CONCLUSION: Further research is needed to track the spread of carbapenem-resistant OXA-type lactamase genes from *A. baumannii* in hospital settings, as they are becoming a major cause of carbapenem resistance. There should be effective infection control systems in place, as well as tight regulations governing antibiotic use.

KEYWORDS: CRAB**,** Resistance mechanism, CLSI, Molecular characterization, DNA, Conventional PCR

INTRODUCTION:

Acinetobacter baumannii is a Gram-negative coccobacillus that can easily acquire antibiotic resistance and survive in hospital surroundings [1]. Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is a species of bacteria that is frequently found in the environment, particularly in soil and water. CRAB can cause a variety of diseases, including bacteremia, pneumonia, urinary tract infection, wound, lung, and other body site infections. The bacteria are multidrug resistant, making illnesses extremely difficult to treat.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

This organism is considered an opportunistic pathogen responsible for nosocomial infections, especially in intensive care units [2]. *A. baumannii* is a common cause of bacteremia, nosocomial pneumonia or ventilator-associated pneumonia, catheter-related infections, meningitis, peritonitis, skin and wound infections, urinary tract infections, and endocarditis [3]. Its capacity to survive in dry or moist settings at varying pH levels and temperatures allows it to thrive in the hospital setting [1].

*A. baumannii i*s one of the ESKAPE pathogens, along with *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter spp*., which are responsible for the majority of nosocomial infections and are capable of "escaping" the bactericidal activity of antimicrobial agents [4]. The preferred medication for *A. baumannii* is considered to b the carbapenems. However, there has been widespread reporting of rising carbapenem resistance. Both carbapenemase-mediated and non-carbapenemase-mediated resistance in A. *baumannii* are possible. Class A (serine proteases), class B (metallo-beta-lactamases), and class D (oxacillinases) carbapenemases are primarily responsible for carbapenemase-mediated resistance, whereas non-carbapenemase-mediated resistance involves upregulation of the efflux pumps and/or loss of outer membrane porins [5].

Resistance to carbapenem in *A. baumannii* is most frequently due to oxacillinases, which can be intrinsic or acquired.

Since the intrinsic blaOXA-51 gene is found on *A. baumannii's* chromosome, the organism is thought to be unique to it. In contrast to MBLs encoded by the blaIMP, blaVIM, blaNDM, and blaSIM genes, acquired OXA enzymes, which are produced by the blaOXA-23, blaOXA-40, and blaOXA-58 genes, are more prevalent in A. baumannii isolates [6].

The overproduction and proliferation of OXA genes in A. baumannii are mostly caused by the insertion sequence. Insertional sequences that act as promoters for OXA gene overexpression are a primary cause of carbapenem resistance. The resistance genes blaOXA-23, blaOXA-51, and blaOXA-58 have all been related to ISAba1, an IS4 family insertion sequence [7]. A.baumannii can develop resistance to a wide range of routinely used antimicrobial drugs [9 ,10] Carbapenems were formerly considered a last choice for treating infections caused by MDR, Gram-negative bacteria, however carbapenem resistance has recently become more widespread in A. baumannii. Multi and extensively drug-resistant (MDR and XDR) Acinetobacter baumannii (A. baumannii)

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

are two principal causal organisms of nosocomial infections, resulting to increased morbidity and mortality, which have been gradually growing internationally over the last decade [8,9].

Several resistance mechanisms of *A. baumannii* against carbapenems have been reported, including antimicrobial-inactivating enzymes, efflux pump, loss of the CarO outer membrane porin, and decreased target access [10,11]. One of the most important carbapenem resistance mechanisms is the production of class D β-lactamases (oxacillinase; OXA). This group of enzymes can hydrolyze oxacillin and the third-generation cephalosporins, but possesses weak activity against carbapenems [12]. Carbapenems are extremely effective for treating infections caused by Gram-negative bacilli that are resistant to other b-lactam antibiotics. However, carbapenem-resistant Gram-negative bacilli are increasingly being isolated from clinical samples. Since the early 1990s, Pseudomonas aeruginosa, Acinetobacter baumannii, and a variety of

Enterobacteriaceae species expressing IMP-type metallo-b-lactamases have been discovered in many Japanese hospitals, with identical or comparable enzymes later discovered abroad.

Different mechanisms may confer carbapenem resistance in *Acinetobacter* spp., but production of carbapenemases is considered the most important one, particularly those belonging to Ambler's class D, also known as oxacilinases (OXA). The class B carbapenemases or metallo-β-lactamases (MβLs) can also be found among *Acinetobacter* spp., although less frequently.

The presence of the OXA-23 subtype is considered the most common mechanism for *A. baumannii* resistance to the carbapenems . OXA-51-like is intrinsic to *A. baumannii*, and its detection is a convenient method for the identification of this species . OXA-24 shares 60% amino acid identity with OXA-23 [13]. The OXA-type carbapenems identified in *A. baumannii* include both the acquired types (OXA-23-, OXA-24/40- and OXA-58-like), where their gene clusters are either in the chromosome or plasmid , and the naturally occurring chromosomal OXA-51-like . Studies on the genomic sequences surrounding these genes revealed that insertion sequence (IS) elements play a critical role in the expression of several OXA genes in A. baumannii [9, 14]. The IS is a short DNA sequence that functions as a simple transposable element and can contain resistance genes [15]. The presence of ISs such as ISAba-1, ISAba-4, and ISAba1-25, which encode transposases upstream of blaOXA genes and provide promoter sequences, enhances the expression and transformation of the OXA genes [16]. Studies have revealed that IS*Aba-1*, from the IS4 family, is found upstream of $bla_{\text{OXA-51}}$, $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-58}}$ genes

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

in *Acinetobacter* species [16]. The presence of an upstream ISAba-1 sequence may influence carbapenem resistance mediated by blaOXA-like genes [16].

Five major groups of OXA with carbapenemase activity have been identified in *Acinetobacter baumannii*: acquired OXA-23-like, OXA-40-like, OXA-58-like, and OXA-143-like families, and the OXA-51 group, which codifies a chromosomal oxacilinase intrinsic to *A. baumannii*. When overexpressed, these enzymes can confer carbapenem resistance [15].

Among OXA, the variants comprising the OXA-23-like family have been detected throughout the world, and have also been pointed out as the predominant carbapenamases among *Acinetobacter* in several geographic regions.

Therefore, the present study was undertaken to study the molecular characterization of invasive carbapenem-resistant *Acinetobacter baumannii* with special reference blaOXA-23gene in ICU patients.

MATERIAL AND METHODS:

This was a Crosssectional study carried out in the Department of Microbiology for a period of 1 year i.e, March 2023 to March 2024 at a tertiary care centre. A total of 130 non-duplicate, consecutive, carbapenem-resistant isolates recovered from ICU patients of *Acinetobacter* species were included in this study. The isolates were obtained from invasive clinical specimens including blood, endotracheal aspirates (ETAs) and the tissue. The isolates were identified up to the species level as *Acinetobacter baumannii* by standard biochemical tests and the Antimicrobial susceptibility testing was performed according to the CLSI guidelines 2023. The DNA was extracted using the Qiagen DNA extraction kit from the clinical samples where the confirmation of the gene OXA-23 gene was done by the conventional PCR [17]. The Antimicrobial Susceptibility Testing The antimicrobial susceptibility profiles of clinical isolates were determined by disk-diffusion, according to the guidelines of the Clinical and Laboratory Standards Institute .The following antimicrobials (CECON – São Paulo, Brazil) were tested: amikacin (30μg), ampicillin/sulbactam (10μg), cefepime (30μg), ceftazidime (30μg), ciprofloxacin (5μg), gentamicin (10μg), imipenem (10μg), meropenem (10μg), piperacillin/tazobactam, (75mg/10μg), sulfamethoxazole/trimethoprim (23.75/1.25μg), ticarcillin/clavulanate (75/10μg), and tetracycline (30μg). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains [17].

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

The Phenotypic Detection method

CarbAcineto NP test was used for carbapenemase phenotypic detection. All of the research isolates that needed to be evaluated were cultivated for 24 hours on a Mueller-Hinton agar plate, and the isolated colonies were then re-suspended in two 1.5 ml centrifuge tubes (A and B) containing 100 l NaCl (5 M). 100 l of solution A (phenol red solution with zinc sulphate) and 100 l of solution A with imipenem (6 mg/ml) were added to tubes A and B, respectively. Maximum 2 hours were allowed for the tubes to be incubated at 37 °C. The hydrolysis of imipenem caused a pH value reduction, which caused a colour shift in tube B, indicating the presence of carbapenemase [17-18]. BAA-1705 and BAA-1706 were simultaneously listed as positive and negative controls, respectively.

The Molecular Characterization of the Gene by phenotypic method

The DNA was isolated using the Qiamp DNA Blood Mini Kit (QIAGEN, Germany) as per the manufactures guidelines. The extracted DNA and the gene was confirmed by the PCR to detect the presence of acquired OXA genes namely OXA-23.

The DNA was eluted in 60 µl elution buffer and preserve at -20 °C till PCR analysis. For amplification of the target gene, PCR was carried out in a 50 µL reaction mixture with 30 no. of cycles. The primers were purchased from "**Saha gene'** and was reconstituted with sterile double distilled water based on the manufacturer's instruction.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

Figure No.1: The DNA Extraction kit **Figure No.2: The Reagents used for the DNA Extraction**

Fragment	Gene	Primer sequence	Length (bp)	Reference
A	OXA-23 FA OXA-23-RA	5'- ATTTCTGACCGCATTTCCAT-3' 5'- GGTTAGTTGGCCCCCTTAAA-3'	501 bp	19

Table No. 1 : Primers used to amplify OXA-23 gene fragments.

Figure No. 3: The OXA-23 primer from the Saha gene

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

Polymerase Chain Reaction (PCR)

For the PCR amplification, 2 µl of template DNA was added to 18 µl reaction containing 10 µl of Qiagen master mix, 2 µl of primer mix (1 µl each of the respective forward and reverse primers) and 6 µl of molecular-grade water[20].

The PCR cycling conditions

Step	**Program** **OXA-23** **Time** **Temperature**	**Cycles**
Initial denaturation	15 min 95 ºC	
Denaturation	30 s 94 ºC	
Annealing	1min 30 s 52 ºC	
Extension	1 min 30 s 72º C	30
Final extension	1 min 30 s 72º C	

Table No. 2 : The PCR cycling conditions to amplify OXA-23 gene fragments

For acquired OXA genes, the initial denaturation was at 95 °C for 15 min, 30 cycles of 94 °C for 30 s, 52 °C for 1 min 30 s and 72 °C for 1 min 30 s, followed by extension of 72 °C for 1 min 30 s.

The Agarose gel preparation and visualized by Gel Doc™ EZ Gel Documentation System

The Agarose Gel Electrophoresis was performed in order to identify the Purified PCR Product which was previously identified by its amplified DNA fragments. The resulting PCR product was subjected to 1 % agarose gel electrophoresis and visualized by Gel Doc™ EZ Gel Documentation

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

System (Bio-Rad Laboratories Inc., Hercules, CA, USA). A 1 kb DNA Ladder (Thermo Fisher Scientific ™, Waltham, MA, USA) was used as the marker to evaluate the PCR product of the sample [21].

RESULTS

In the present study a total of 1216 clinical samples were included out of which 130 invasive clinical isolates of Acinetobacter species were studied. The maximum number of isolates were from the ETA samples with 98 (75%), 25 (19.2%) from blood and 7 (5.3%) from the tissue [Table 4]. The ratio of Males 78(60%) was more as compared to that of the Females 52 (40%) with the maximum age of 31-40 being affected the most followed by 41-50 and least in the age group above 61 years of age.

Type of Clinical Isolates	**Number of Ioslates**	**Percentage**
Acinetobacter species	130	10.6%
Others clinical isolates	1086	89.3%

Table No. 3: Total Number of clinical isolates

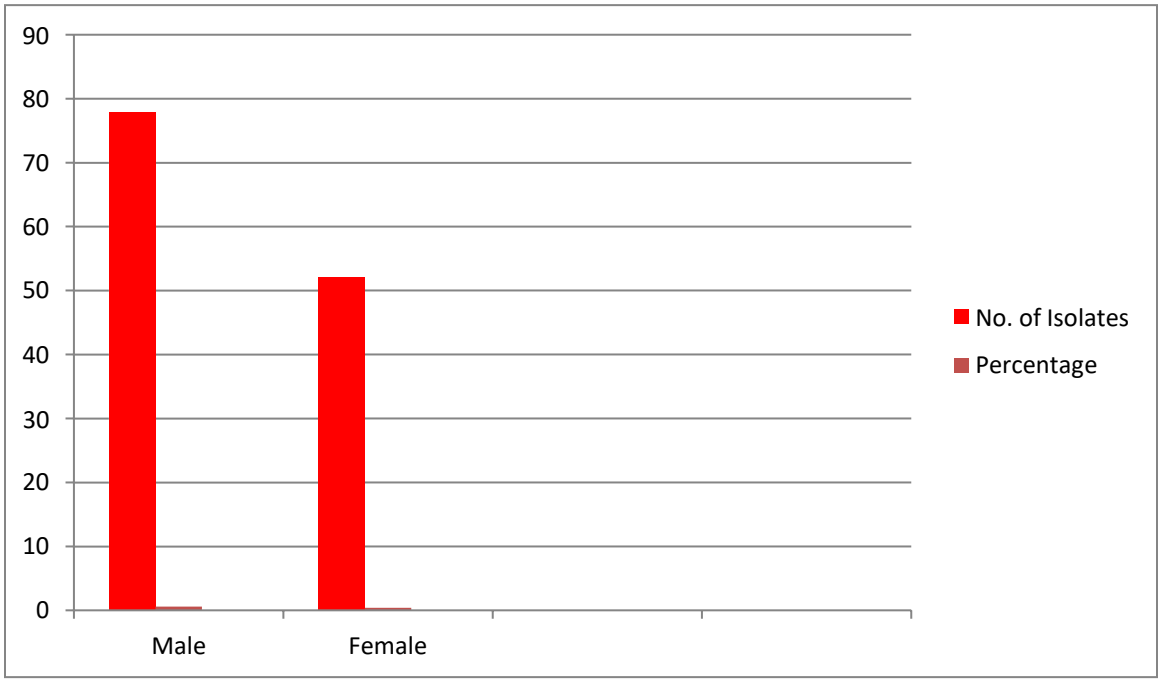
Type of isolates	**Number of Isolates**	**Percentage**
ETA	98	75%
Blood	25	19.2%
Tissue	7	5.3%
Total	**130**	**100%**

Table No. 4: Total Number of clinical isolates of Acinetobacter species

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

Gender	Total no. of Cases studies (N=130)	Percentage
Male	78	60%
Female	52	40%

Table No. 5 : Genderwise distribution of the *Candida albicans*



Graph No. 1: The graphical Respresentation of the Genderwise distribution

The ratio of Males 78 (60%) was more as compared to that of the Females 52(40%) [Table No. 5] with the maximum age of 31-40 being affected the most followed by 41-50 and least in the age group above 61 years of age [Table No. 6]. There was only 1 *Acinetobacter baumannii* isolated in the age group of 0-10 years of age.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

S.No.	Age (in years)	No. of Cases	Percentage
1.	0- 10	1	0.7%
2.	11-20	8	6.1 %
3.	21-30	16	12.3%
4.	31-40	62	47.6%
5.	41-50	25	19.2 %
6.	51-60	13	10%
7.	≥61	5	3.8%

Table No.6 : Age wise distribution of *A.baumannii* patients from the study

Antimicrobial susceptibility testing revealed that all the isolates were resistant to ceftazidime, cefepime, piperacillin/tazobactam, cefoperazone/sulbactam, aztreonam, imipenem, meropenem, amikacin, netilmycin, tetracycline, tobramycin, levofloxacin and co-trimoxazole. In the present study it was also observed that around 126 (96.9%) of the study isolates were susceptible to polymyxin B (colistin).

Among the 130 clinical isolates, the CarbAcineto NP test was positive in 122(93.8%) isolates and negative in 8 (7.27%)

The Molecular characterization for the detection of the genes in Acinetobacter was performed where the DNA was isolated using the Qiagen DNA extraction kit as per the manufacture's guidelines. The PCR was run for the detection OXA Gene.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

Figure No. 4: The PCR cycling conditions

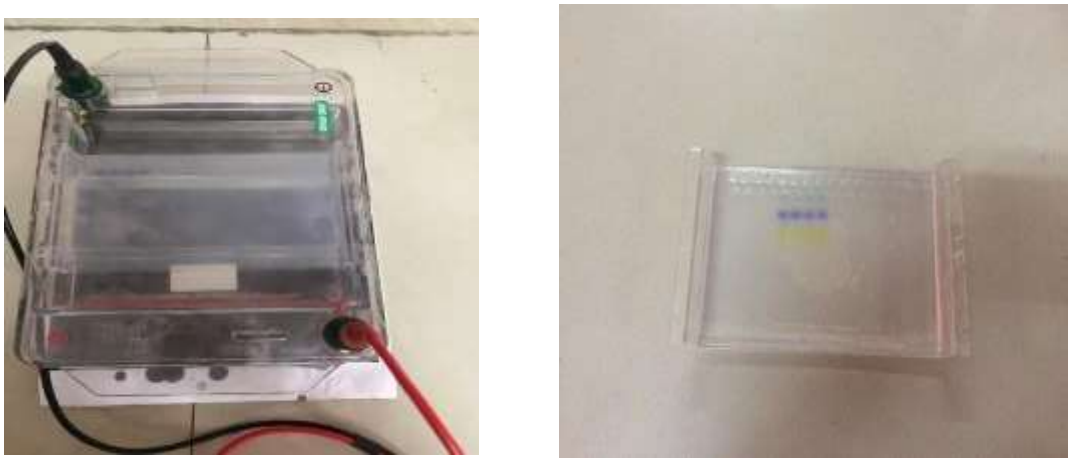


Figure No. 5: Electrophoresis unit under run Run of Amplified product

The Molecular characterization reveals that among the 130 isolates tested. blaOXA-23 gene was present in all the 130 isolates (100%).

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

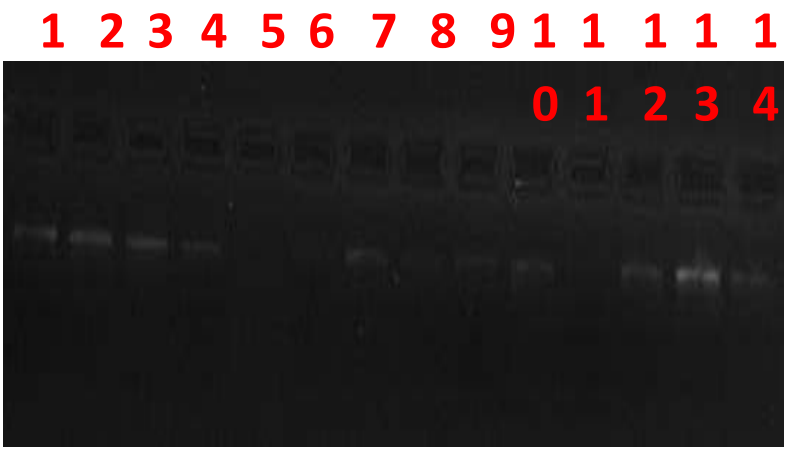


Figure No.6: The DNA Extraction of the OXA resistant OXA-23 gene

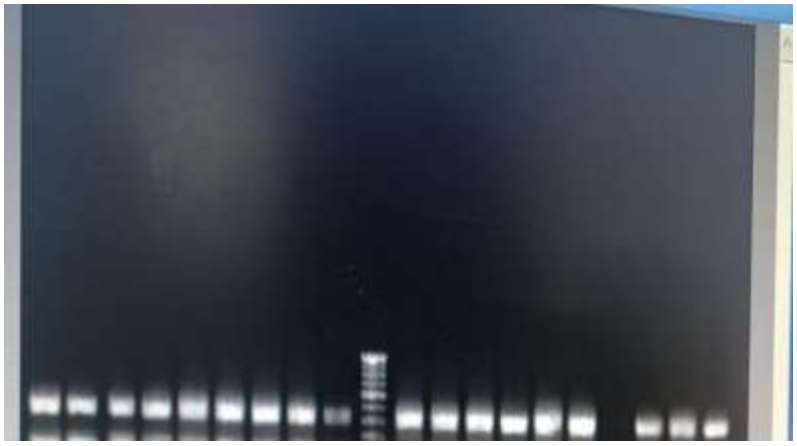


Figure No.7 : The Amplified DNA with PCR for OXA-23 gene of *A. baumannii* . Lane -1-9 are positive for OXA-23 gene; Lane 10 is the DNA Ladder; Lane 11-16 and 19, 20 are OXA-23 gene positive; Lane 17 is the Negative control for OXA-23 gene; Lane 18 is the positive control for OXA-23 gene

The acquired OXA carbapenemase, blaOXA-23 gene was present in all the 130 isolates (100%). The molecular detection of the blaoxa-23-like gene revealed a 501 bp band in all clinical isolates, which preliminarily confirmed the identification of the clinical isolates as being *A. baumannii*.

DISCUSSION

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

Worldwide infections associated with healthcare are increasingly being reported as multidrug-resistant A. baumannii. As a result, carbapenems are the chosen antibiotic for treating severe MDR A. baumannii infections. There are several resistance mechanisms emerging, including class D, class B metallo-b-lactamases, and OXA carbapenemases, which have a significant impact on the evolution of A. baumannii carbapenem resistance worldwide [12].

The detection of *A. baumannii* producing OXA-23 is frequent in many countries, suggesting a worldwide dissemination of this enzyme. According to Kempf and Rolain, Europe, Singapore, Australia, the USA, Algeria, Egypt, Libya, South Africa, Thailand, Tunisia, Iraq, and French Polynesia reported either nosocomial outbreaks or sporadic cases of *Acinetobacter* producing OXA-23. In Asia, the most prevalent disseminated class D enzyme amongst *A. baumannii* in China and Korea is OXA-23. In Latin America, OXA-23 was identified in *A. baumannii* isolates from Argentina, Colombia, and Brazil. In Brazil, OXA-23-like enzymes have been identified as one of the major carbapenem resistance mechanism in *Acinetobacter* spp. In Rio de Janeiro, a surveillance study conducted in eight hospitals showed a wide dissemination of isolates producing such carbapenemases. In Niterói, the occurrence of *A. baumannii* strains carrying *bla*OXA-23 has already been reported at a public hospital, however, at a smaller rate (15%) than found in the present study [22-24].

In the present study a total of 1216 clinical samples were included out of which 130 invasive clinical isolates of Acinetobacter species were studied. The prevalence of Acinetobacter was found to be 10.6 %. This study was similar to the study performed by Sharma RK et al. where the percentage of Acinetobacter isolates was found to be (6.42%) [25]. There were other studies which were also parallel to our study stating the rate of Acinetobacter to be similar studies by Fayyaz et al [26] (10.9%) and Goossens [27] (4.9%) but in contrast with the study by Sabir et al, where the percentage of positive culture was found to be 87.17%, which was much higher than the present study [28].

The ratio of Males 78(60%) was more as compared to that of the Females 52 (40%) with the maximum age of 31-40 being affected the most followed by 41-50 and least in the age group above 61 years of age. This study was in support with the study performed by the other authors, where the rate of male (75.36%) and female (24.28%) was observed [25]. Another study was also found

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

to be similar in the studies by Fayyaz et al [26] but in contrast with the studies by Tahseen and Talib and Saleem *et al*. [29, 30].

The maximum number of isolates were from the ETA samples with 98 (75%), 25 (19.2%) from blood and 7 (5.3%) from the tissue. This study was supported by Sharma RK et al. and L Dortet et al.[25] [19] where similar findings were recorded.

In the present study antimicrobial susceptibility testing revealed that all the isolates were resistant to ceftazidime, cefepime, piperacillin/tazobactam, cefoperazone/sulbactam, aztreonam, imipenem, meropenem, amikacin, netilmycin, tetracycline, tobramycin, levofloxacin and co-trimoxazole. In the present study it was also observed that around 126 (96.9%) of the study isolates were susceptible to polymyxin B (colistin). This study was similar to the study by the author authors where polymyxin B was observed to be susceptible [25] [19]. Among the 130 clinical isolates, the CarbAcineto NP test was positive in 122(93.8%) isolates and negative in 8 (7.27%)

This study was similar to the study by the other author Vijaykumar S et al in 2016 [20]. It was noted that around 108 (98.1%) of the study isolates were susceptible to polymyxin B and colistin, which was parallel to the study performed by the other author [20].

In the present study among the study isolates, OXA carbapenemases were detected in all 130 (100%) isolates of carbapenem-resistant *A. baumannii*. The blaOXA-23-like oxacillinases were the most common type. This study was supported by the study from East India also showed the OXA-23 genes as the prevalent type of oxacillinase contributing to carbapenem resistance [31].
The study by other investigator were consistent with those of Yang et al, which observed 100% positivity in Modified Hodge Test among *A. baumannii* strains that were IMP-R and OXA-23 producers [33]. The study by another investigator was also in accordance to the current study where 81 isolates (95.4%) carried the *bla*OXA-23 gene [34].
Studies have reported that carbapenem resistance in *A. baumannii* is mainly due to carbapenemase mediated. However, non-carbapenemase-mediated resistance mechanisms such as reduced membrane permeability due to porin changes and overexpression of efflux pumps make a trivial contribution toward carbapenem resistance in *A. baumannii* [35-38].

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

Acinetobacter species is a new global illness that is acquired in hospitals. Because A. baumannii drug resistance is a major concern in today's healthcare settings, effective infection control techniques and strict antibiotic use regulations should be implemented. Further research is needed to monitor the dissemination of carbapenem-resistant OXA-type _-lactamase genes from A. baumannii in hospital settings, as they are becoming a substantial source of carbapenem resistance.

CONCLUSION

A high frequency of *bla*OXA-23 (100%) was observed among isolates analyzed. This result implies that production of OXA-23-like enzymes may be an important mechanism of carbapenem resistance among isolates present in the hospital studied.

It could be beneficial to routinely perform the surveillance of isolates producing carbapenemases in the hospital investigated. In addition, strict control measures, as well as prudent utilization of antimicrobials agents should be continuously promoted.

Declarations:

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: We have consent to participate.

Consent for publication: We have consent for the publication of this paper.

Authors' contributions: All the authors equally contributed the work.

REFERENCES :

1. E. Girão, A.S. Levin, M. Basso, *et al*. Trends and outcome of 1121 nosocomial bloodstream infections in intensive care units in a Brazilian hospital, 1999–2003. Int J Infect. 2008; 12 :pp. 145-146

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

2. Tosi, M, Roa, E, De Biasi S, Munari E, Venturelli S, Coloretti, I, Biagioni E, Cossarizza A, Girardis, M. Multidrug resistant bacteria in critically ill patients: A step further antibiotic therapy. J. Emerg. Crit. Care Med. 2018; 2, 103.

3. Moubareck, C.A, Halat, D.H. Insights into Acinetobacter baumannii: A review of microbiological, virulence, and resistance traits in a threatening nosocomial pathogen. Antibiotics. 2020; 9, 119.

4.. Paiboonvong T, Rodjun V, Houngsaitong J, Chomnawang M, Montakantikul P, Chulavatnatol S. Comparative in vitro activity of sitafloxacin against multidrug-resistant and carbapenem-resistant Acinetobacter baumannii clinical isolates in Thailand. Pharm. Sci. Asia. 2020; 47, 37–42.

5. Mulani, M.S.; Kamble, E.E, Kumkar, S.N, Tawre, M.S, Pardesi, K.R. Emerging strategies to combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. Front. Microbiol. 2019; 10, 539.

6. A.H. Torres, E.G. Vázquez, G. Yagüe, J.G. Gómez. *Acinetobacter baumannii* multirresistente: situación clínica actual y nuevas perspectivas. Rev Esp Quimioter. 2010; 23: pp. 12-19

7. Kim UJ, Kim HK, An JH, Cho SK, Park K-H, Jang H-C. Update on the epidemiology, treatment, and outcomes of carbapenem-resistant *Acinetobacter infections*. *Chonnam Med J.* 2014; 50(2):37.

8. Seifert, H, Stefanik, D, Olesky, M, Higgins, P.G. In vitro activity of the novel fluorocycline TP-6076 against carbapenem-resistant Acinetobacter baumannii. Int. J. Antimicrob. Agents. 2020; 55:105829.

9. Lee, C.R, Lee, J.H, Park, M, Park, K.S, Bae, I.K, Kim, Y.B, Cha, C.J., Jeong, B.C., Lee, S.H. Biology ofAcinetobacter baumannii: Pathogenesis, antibiotic resistance mechanisms, and prospective treatment option.Front. Cell Infect. Microbiol. 2017, 7: 55.

10 . Seifert, H, Stefanik, D, Olesky, M, Higgins, P.G. In vitro activity of the novel fluorocycline TP-6076 against carbapenem-resistant Acinetobacter baumannii. Int. J. Antimicrob. Agents. 2020; 55:105829.

11. El-Badawy M.F, Abdelwahab S.F, Alghamdi S.A, Shohayeb M.M. Characterization of phenotypic and genotypic traits of carbapenem-resistant Acinetobacter baumannii clinical isolates recovered from a tertiary care hospital in Taif, Saudi Arabia. Infect. Drug Resist. 2019, 12: 3113–3124.

12. Li S, Duan X, Peng Y, Rui Y. Molecular characteristics of carbapenem-resistant Acinetobacter spp. from clinical infection samples and fecal survey samples in Southern China. *BMC Infect Dis*. 2019; 19(1):1–12.

13. Elshamy A.A, Aboshanab K.M. A review on bacterial resistance to carbapenems: Epidemiology, detection and treatment options. Future Sci. OA 2020, 6, FSO438.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

14. C.S. Antonio, P.R. Neves, M. Medeiros, E.M. Mamizuka, M.R. Elmor de Araújo, N. Lincopan. High prevalence of carbapenem-resistant *Acinetobacter baumannii* carrying the *bla*OXA-143 gene in brazilian hospitals. Antimicrob Agents Chemother. 2011; 55 : pp. 1322-1323

15. Paiboonvong T, Rodjun V, Houngsaitong J, Chomnawang M, Montakantikul P, Chulavatnatol S. Comparative in vitro activity of sitafloxacin against multidrug-resistant and carbapenem-resistant Acinetobacter baumannii clinical isolates in Thailand. Pharm. Sci. Asia. 2020; 47, 37–42.

16. Lin M.F, Lan C.Y. Antimicrobial resistance in Acinetobacter baumannii: From bench to bedside. World J. Clin. Cases. 2014; 2: 787–814.

17. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: twenty second informational supplement M100–S22. Wayne: CLSI; 2022

18. Dortet L, Poirel L, Errera C, Nordmann P. Carbacineto NP test for rapid detection of carbapenemase-producers in Acinetobacter spp. J Clin Microbiol. 2014;10.1128/JCM.00594-14.

19. Krit Thirapanmethee , Thayapa Srisiri-a-nun , Jantana Houngsaitong , Preecha Montakantikul , Piyatip Khuntayaporn and Mullika Traidej Chomnawang. Prevalence of OXA-Type _-Lactamase Genes among Carbapenem-Resistant Acinetobacter baumannii
Clinical Isolates in Thailand. Antibiotics. 2020; 9, 864; doi:10.3390/antibiotics9120864

20. Saranya Vijayakumar , Radha Gopi , Priya Gunasekaran , Manjurekar Bharathy ,Kamini Walia , Shalini Anandan , Balaji Veeraraghavan. Molecular Characterization of Invasive Carbapenem-Resistant Acinetobacter baumannii from a Tertiary Care Hospital in South India. Infect Dis Ther . 2016; 5:379–387.

21.Aneta K, Urbanek 1 , Zofia Łapi ´ nska , Daria Derkacz 1 and Anna Krasowska . The Role of ERG11 Point Mutations in the Resistance of Candida albicans to Fluconazole in the Presence of Lactate. Pathogens 2022; 11, 1289.

22. Y. Kouyama, S. Harada, Y. Ishii, *et al*. Molecular characterization of carbapenem-non-susceptible *Acinetobacter* spp. in Japan: predominance of multidrug-resistant *Acinetobacter baumannii* clonal complex 92 and IMP-type metallo-β-lactamase-producing non-*baumannii Acinetobacter* species. J Infect Chemother. 2012 ; pp. 14

23. V.A. Gundi, L. Dijkshoorn, S. Burignat, D. Raoult, B. La Scola. Validation of partial rpoB gene sequence analysis for the identification of clinically important and
emerging *Acinetobacter* species. Microbiology. 2009; 55 : pp. 2333-2341

24. F. Rossi. The challenges of antimicrobial resistance in Brazil. Clin Infect Dis. 2011; 52 : pp. 1138-1143

25. Rajesh K Sharma, Ved P Mamoria. A Prospective Study on Prevalence and Antibiotic Susceptibility Pattern of Acinetobacter baumannii in Clinical Samples obtained from Patients admitted in Various Wards and Intensive Care Units. Journal of Mahatma Gandhi University of Medical Sciences and Technology. 2017; 2(3):122-127

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 06, 2024

26. Fayyaz M Khan IU, Hussain A Mirza IA Ali S, Akbar N. Frequency and antimicrobial susceptibility pattern of Acinetobacter species isolated from pus and pus swab specimens. J Coll Physicians Surg Pak .2015; 25(5):346-349.

27. Goossens H. MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) results from Europe: comparison of antibiotic susceptibilities between countries and centre types. J Antimicrob Chemother 2000 ;46(Suppl T2):39-52.

28. Sabir R, Alvi FD, Fawwad A. Antimicrobial susceptibility pattern of aerobic microbial isolates in a clinical lab in Karachi— Pakistan. Pak J Med Sci. 2013; 29(3):851-855.

29 Tahseen U, Talib MT. Acinetobacter infections as an emerging threat in intensive care units. J Ayub Med Coll Abbottabad. 2015; 27(1):113-116.

30. Saleem AF, Shah MS, Shaikh AS, Mir F, Zaidi AK. Acinetobacter species meningitis in children: a case series from Karachi, Pakistan. J Infect Dev Ctries .2011; 5(11):809-814.

32. Saranathan R, Vasanth V, Vasanth T, Shabareesh PRV, Shashikala P, Devi CS, et al. Emergence of carbapenem non-susceptible multidrug resistant Acinetobacter baumannii strains of CC 103B and CC 92B clonal complexes harboring OXA-type carbapenemases and metallo-b-lactamases in Southern India. Microbiol Immunol. 2015;59(5):277–84.

33. H.Y. Yang, H.J. Lee, J.T. Suh, K.M. Lee. Outbreaks of imipenem resistant *Acinetobacter baumannii* producing OXA-23 β-lactamase in a tertiary care hospital in Korea. Yonsei Med J, 50. 2009; pp. 764-770

34. Laís Lisboa Corrêa. Detection of *bla*OXA-23 in *Acinetobacter* spp. isolated from patients of a university hospital. 2012; 16(6):521-526.

35. Amudhan SM, Sekar U, Arunagiri K, Sekar B. OXA beta-lactamase-mediated carbapenem resistance in Acinetobacter baumannii. Indian J Med Microbiol. 2011; 29(3):269–74.

36. Evans BA, Hamouda A, Amyes SGB. The rise of carbapenem-resistant Acinetobacter baumannii. Curr Pharm Des. 2013; 19:223–38.

37. M. Kempf, J.M. Rolain. Emergence of resistance to carbapenems in *Acinetobacter baumannii* in Europe: clinical impact and therapeutic options. Int J Antimicrob Agents. 2012; 39 pp. 105-114

38. G.H. Furtado, A.C. Cavalcante, E.A. Medeiros, A.C. Gales, V.G. Oliveira, R. Girardelo. Bloodstream infections with OXA-23-producing *Acinetobacter baumannii* isolates in a university-affiliated hospital in Brazil: epidemiology and clinical outcomes. Am J Infect Control, 39 (2011), pp. 706-708