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

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ABSTRACT

INTRODUCTION: Carbapenem-resistant Acinetobacter baumannii (CRAB) is a significant health concern in the treatment of infectious disorders. Traditionally, carbapenems were used as a last resort to treat MDR Gram-negative bacteria infections. A. baumannii has recently demonstrated a rise in carbapenem resistance. The majority of carbapenem resistance mechanisms in A. baumannii are due to enzymatic breakdown by -lactamases.

AIM AND OBJECTIVES: To study the Molecular Characterization of Invasive Carbapenem-Resistant *Acinetobacter baumannii* with special reference to blaVIM 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, April 2023 to April 2024 at a tertiary care centre. A total of 120 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

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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 blaVIM was detected using the conventional PCR assay.

RESULTS: In the present study a total of 1034 clinical samples were collected in which 120 Acinetobacter species were isolated. The maximum number of isolates were from the ETA samples with 91 (75.8%), 24 (20%) from blood and 5 (4.1%) from the tissue. The ratio of Males 75(62.5%) was more as compared to that of the Females 45 (37.5%) 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. 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 116 (96.6%) of the study isolates were susceptible to polymyxin B (colistin).

The Molecular characterization reveals that among the 120 isolates tested for MBL, the twelve isolates were positive for the blaVIM gene (10%).

CONCLUSION: Further studies are necessary to monitor the spread of carbapenem-resistant OXA-type lactamase genes from *A. baumannii* in hospital settings since they are becoming a significant cause of carbapenem resistance. There should be efficient infection control procedures and strict regulation on the use of Antibiotics followed.

KEYWORDS: CRAB**,** Resistance mechanism, Carbapenem, 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.

In *Acinetobacter* spp., β-lactamases are the most common contributors to resistance to extended-spectrum cephalosporins and carbapenems, including extended-spectrum β-lactamases (ESBL), metallo-β-lactamases (MBL), carbapenem-hydrolyzing class D β-lactamases (CHDL),

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and *Acinetobacter*-derived cephalosporinases (ADC) [2].

Carbapenem resistance in *A. baumannii* is mainly due to Class D and Class B carbapenemases belonging to Ambler's classification of β-lactamases. Although various mechanisms contribute to carbapenem resistance, the majority is due to class D carbapenemases such as *bla*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-24-like}}$ and *bla*$_{\text{OXA-58-like}}$ *bla*$_{\text{OXA-23-like}}$ producing *A. baumannii* responsible for causing outbreaks have been reported from various regions of the world[7]. Class D carbapenemase in transposons, have the ability to rapidly spread in successful clonal lineages of *A. baumannii*.

This organism is considered an opportunistic pathogen responsible for nosocomial infections, especially in intensive care units [2]. *A. baumannii* commonly causes bacteremia, nosocomial-acquired pneumonia or ventilator-associated pneumonia, catheter-related infections, meningitis, peritonitis, skin and wound infections, urinary tract infections, and endocarditis [3]. The ability to survive in dry or moist conditions at various pH levels and temperatures renders it able to grow in the hospital environment [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 [4,5].

Carbapenems are thought to be the most effective treatment for *A. baumannii*. However, there has been widespread reporting of increased carbapenem resistance. *A. baumannii* resistance can be carbapenemase-mediated or non-carbapenemase-mediated. Carbapenemase-mediated resistance is typically caused by class A (serine proteases), class B (metallo-beta-lactamases), and class D (oxacillinases) carbapenemases, whereas non-carbapenemase-mediated resistance involves overexpression of efflux pumps and/or loss of outer membrane porins [6].

The β-lactamases and resistance determinants were detected by PCR including Ambler class A ESBL genes (*bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$, *bla*$_{\text{CTX-M}}$, *bla*$_{\text{VEB}}$, and *bla*$_{\text{PER}}$), Amber class B MBL genes (*bla*$_{\text{IMP}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{GIM}}$, *bla*$_{\text{SPM}}$, and *bla*$_{\text{SIM}}$), an Amber class C AmpC β-lactamase genes (*bla*$_{\text{ADC}}$), Amber class D carbapenemase genes (*bla*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA24-like}}$, *bla*$_{\text{OXA-51-like}}$, and *bla*$_{\text{OXA-58-like}}$), integrase genes (*intI1*, *intI2*, and *intI3*), IS elements (IS*Aba1*, IS*Aba2*, IS*Aba3*, and IS*18*), transposase genes, and a putative replicase gene, *repAci1* 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

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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 [7-10].

A.baumannii can develop resistance to many classes of commonly used antimicrobial agents [9 ,10] where Carbapenems was considered as a last resort to treat infections caused by MDR, Gram-negative bacteria, but recently, carbapenem resistance has been increasingly common in *A. baumannii.* The Multi and extensively drug-resistant (MDR and XDR) *Acinetobacter baumannii* (*A. baumannii*) are two main causative agents of nosocomial infections leading to increased morbidity and mortality which have been progressively increasing globally over the last decade [11,12,13].

A. baumannii has been shown to have a variety of carbapenem resistance pathways, including antimicrobial-inactivating enzymes, an efflux pump, deletion of the CarO outer membrane porin, and limited target access [3, 14, 15]. Oxacillinase (OXA), a class D β-lactamase, is a key mechanism of carbapenem resistance. This set of enzymes can hydrolyze oxacillin and third-generation cephalosporins, but they have little activity against carbapenems [16].

Carbapenems are very useful for the treatment of infections caused by Gram-negative bacilli that are resistant to other b-lactam antibiotics. However, carbapenem-resistant Gram-negative bacilli are being isolated with increasing frequency from clinical sources. *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and a number of species of *Enterobacteriaceae* producing IMP-type metallo-b-lactamases have been found in several hospitals in Japan since the early 1990s, and identical or related enzymes have subsequently been detected elsewhere. Thereforethe present study was undertaken to study the molecular characterization of invasive carbapenem-resistant *Acinetobacter baumannii* with special reference to blaVIM gene in ICU patients.

MATERIAL AND METHODS:

This was a Cross sectional study carried out in the Department of Microbiology for a period of 1 year i.e, April 2023 to April 2024. A total of 120 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

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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 blaVIM was done by the PCR [17].

The Antimicrobial Susceptibility Testing

The Susceptibility to different classes of antibiotics was determined by the Kirby Bauer disc diffusion method and interpreted according to the Clinical Laboratory Standard Institute guidelines. Antibiotics tested were ceftazidime (30 µg), cefepime (30 µg), piperacillin/tazobactam (100/10 µg), cefoperazone/Sulbactam (75/30 µg), amikacin (30 µg), netilmycin (30 µg), tobramycin (10 µg), aztreonam (30 µg), levofloxacin (5 µg), tetracycline (30 µg), co-trimoxazole (1.25/23.75 µg), imipenem (10 µg), meropenem (10 µg) and polymyxin B (10 µg) [17] were used as per the CLSI guidelines.

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 [17-19]. BAA-1705 and BAA-1706 were simultaneously listed as positive and negative controls, respectively.

The Molecular Characterization of the Genes 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 the MBL gene blaVIM.

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.

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Figure No.1: The DNA Extraction kit

Fragment	Gene	Primer sequence	Length (bp)	Reference
A	blaVIM-FB blaVIM-RB	5'-CCGATGGTGTTTGGTCGCAT -3' 5'-GAATGCGCAGCACCAGGAA -3'	390bp	20

Table No. 1 : Primers used to amplify blaVIM gene fragments.

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Figure No. 2: The blaVIM primer from the Saha gene

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.

The cyclic conditions for MBL genes, initial denaturation at 95 °C for 15 min, 30 cycles of 94 °C for 30 s, 59 °C for 1 min 30 s and 72 °C for 1 min 30 s were followed by extension of 72 °C for 10 min.

The PCR cycling conditions

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Step	**Progra blaVI**		**Cycles**
	Time	**Temperature**	
Initial denaturation	15 min	95 ºC	
Denaturation	30 s	94 ºC	
Annealing	1 min30 s	59 ºC	30
Extension	1 min 30 s	72º C	
Final extension	10 min	72º C	

Table No. 2 : The PCR cycling conditions to amplify blaVIM gene fragments.

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 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 834 clinical samples were included out of which 120 invasive clinical isolates of Acinetobacter species were studied. The maximum number of isolates were from the ETA samples with 91 (75.8%), 24 (20%) from blood and 5 (4.1%) from the tissue. The ratio of Males 75(62.5%) was more as compared to that of the Females 45 (37.5%) with the maximum

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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. 2].

Type of Clinical Isolates	**Number of Ioslates**	**Percentage**
Acinetobacter species	120	11.16%
Others clinical isolates	914	88.3%

Table No. 3: Total Number of clinical isolates

Type of isolates	**Number of Isolates**	**Percentage**
ETA	91	75.8%
Blood	24	20%
Tissue	5	4.1%
Total	**120**	**100%**

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

Gender	**Total no. of Cases studies (N=120)**	**Percentage**
Male	75	62.5%
Female	45	37.5%

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

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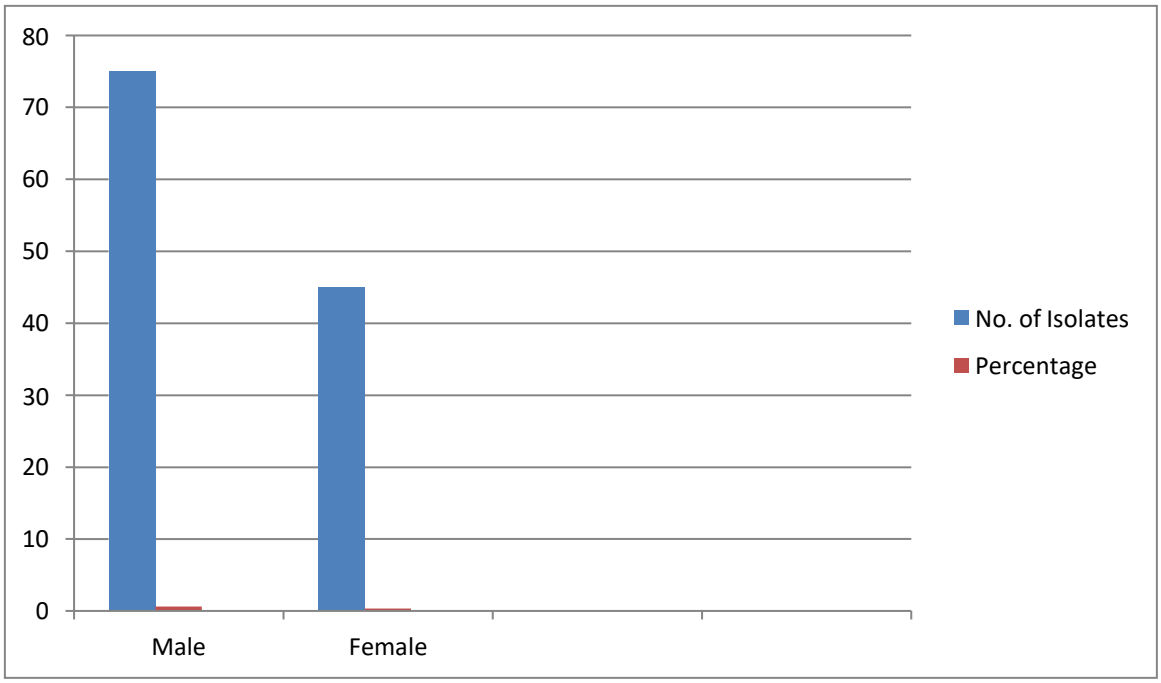

Graph No. 1: The graphical Respresentation of the Genderwise distribution

The ratio of Males 75 (62.5%) was more as compared to that of the Females 45(37.5%) [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 no *Acinetobacter baumannii* isolated in the age group of 0-10 years of age.

S.No.	Age (in years)	No. of Cases	Percentage
1.	0- 10	-	-
2.	11-20	7	5.8 %
3.	21-30	16	13.3 %
4.	31-40	52	43.3%
5.	41-50	26	21.6%
6.	51-60	13	10.8%
7.	≥61	6	5 %

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

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study it was also observed that around 116 (96.6%) of the study isolates were susceptible to polymyxin B (colistin.).

Among the 120 clinical isolates, the CarbAcineto NP test was positive in 112(93.3%) isolates and negative in 8 (6.6%)

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 of MBL Gene.

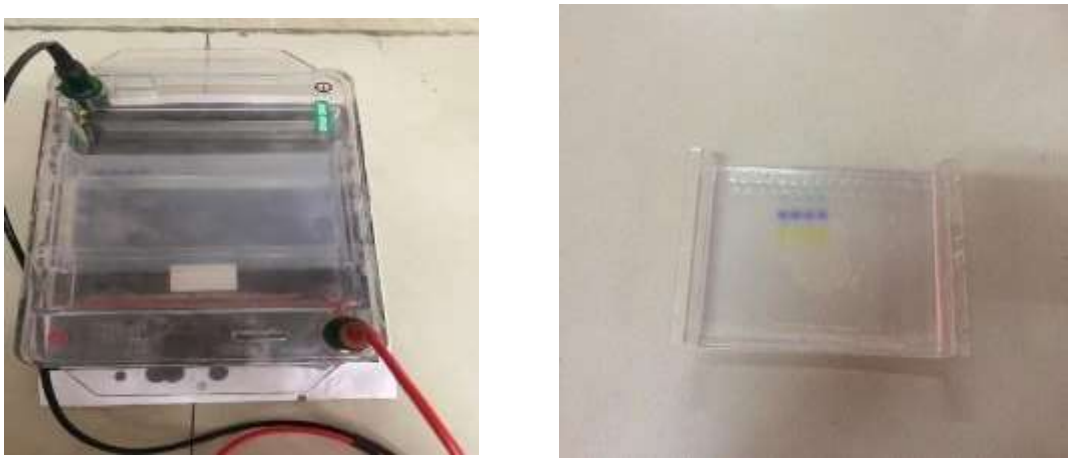

Figure No. 3: Electrophoresis unit under Run of Amplified product

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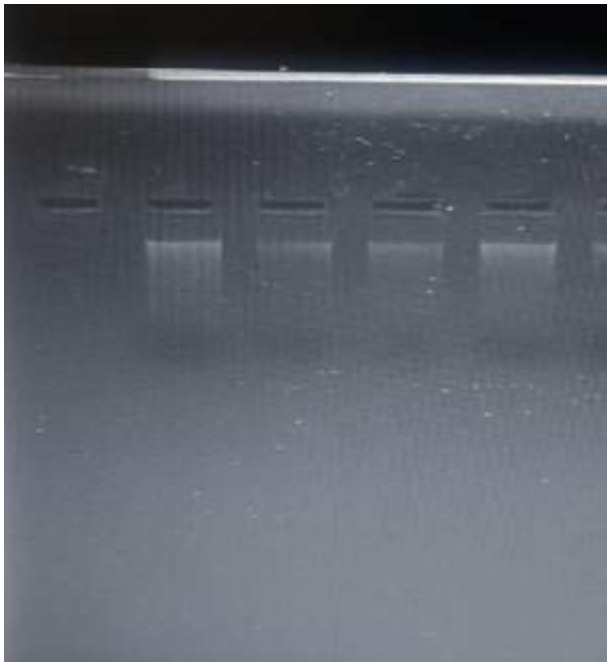

Figure No.4: The DNA Extraction of the MBL resistant blaVIM gene

Figure No. 5: The Amplified DNA with PCR for blaVIM gene of *A. baumannii* . Lane 1 is the positive control; Lane 2 is the sample negative for blaVIM; Lane 3 is the Negative control; Lane 4-7 are sample positive for blaVIM gene; Lane 8 is the DNA Ladder

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Among the 120 isolates tested for MBL, the twelve isolates were positive for the blaVIM gene (10%). The molecular detection of the blaVIM -like gene revealed a 390 bp band in all clinical isolates, which preliminarily confirmed the identification of the clinical isolates as being *A. baumannii.*

DISCUSSION

Globally, 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 [17].

Due to therapeutic challenges, hospital-acquired infections (HAIs) caused by *Acinetobacter baumannii* (HA-AB), particularly carbapenem-resistant strains (HA-CRAB) pose a serious health threat to patients worldwide. *Acinetobacter baumannii* is an opportunistic pathogen of emerging importance in the clinical settings and responsible for up to 20% of infections in ICUs around the globe [21]. The majority of reported clinical cases involved ventilator-associated pneumonia/pulmonary infections, bloodstream infections, skin and soft tissue infections, including burn and surgical wound infections, endocarditis, meningitis, and urinary tract infections. Furthermore, Acinetobacter infections are not limited to hospital settings; reports have surfaced of instances involving apparently healthy individuals of all ages, occurring in community settings, after natural catastrophes, and during wars [22,23]. Treatment of infections caused by this organism is becoming a substantial clinical concern, because A. baumannii has widespread resistance to several of the commonly used antibiotics including cephalosporins, aminoglycosides, quinolones, and carbapenems. *Acinetobacter baumannii* is of particular concern due to its predilection to acquire antibiotic resistance determinants [24].

In the present study a total of 1034 clinical samples were included out of which 120 clinical isolates of Acinetobacter species were studied. The maximum number of isolates were from the ETA samples with 91 (75.8%), 24 (20%) from blood and 5 (4.1%) from the tissue. This study was similar to the study performed by other author Sharma RK et al. where the percentage of

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Acinetobacter isolates was found to be (6.42%) [19, 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 75(62.5%) was more as compared to that of the Females 45 (37.5%) 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. There was no *Acinetobacter baumannii* isolated in the age group of 0-10 years of age. This study was in support with the study performed by the other authors, where the rate of male was more (75.36%) as compared to the female (24.28%) [25]. Another study was also found to be similar in the study by Fayyaz et al [26] but in contrast with the studies by Tahseen and Talib and Saleem et al. [29, 30].

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 116 (96.6%) of the study isolates were susceptible to polymyxin B. This study was similar to the study by the author authors where polymyxin B was observed to be susceptible [25] [19]. It was also noted that around 118 (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].

Among the 120 clinical isolates, the CarbAcineto NP test was positive in 112 (93.3%) isolates and negative in 8 (6.6%). This study was similar to the study by other author Vijaykumar S et al in 2016 [20].

Among the metallo b-lactamases (MBLs), twelve (10%) were positive for the blaVIM gene. However, studies by Saranathan et al. and Amudhan et al. showed IMP-like and blaVIM-like as the prevalent rate of MBL genes to be similar[31].

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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* [32].

Nowadays, the growing drug resistance rate among *A. baumannii* strains is a serious global issue . Resistance mechanisms often involve the development of β-lactamases, such as those found in Ambler classes A, D, and B. These enzymes are commonly linked to mobile genetic elements like plasmids Carbapenem resistance produced by MBL acquisition is thought to be more significant than other resistance mechanisms since MBLs can virtually completely hydrolyze all beta-lactam antibiotics except monobactams [33].

CONCLUSION

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.

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.

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