"DETECTION OF METALLO BETA-LACTAMASES IN CARBAPENEM-RESISTANTACINETOBACTER BAUMANNII BY PHENOTYPIC METHODS''

By

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In

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Under the guidance of

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LIST OF ABBREVIATIONS USED

1) AB : Acinetobacter. Baumannii
2) MDR : Multidrug resistance
3) MBL : Metallo β- lactamases
4) DDST: Double disc synergy test
5) CDT: Combined disc test
6) IMP: Imipenem
7) EDTA :Ethylene diamine tetra acetic acid
8) Et al : et alia

ABSTRACT

Introduction : Acientobacter baumannii is an emerging multidrug resistant opportunistic pathogen that causes a variety of nosocomial infections .in recent years, carbapenem resistance in A.baumannii has increased due to ambler class B Metallo β-lactamases or class D OXA Carbapenemases.

Objective :

1)Isolation and identification of *Acinetobacter baumannii* from various clinical samples.

2)To know the antibiotic susceptibility pattern of of *Acinetobacter baumannii* isolates.

3)Comparison of phenotypic tests for the detection of MBL in *Acinetobacter baumannii Isolates.*

Materials and materials:

Ninety one *Acinetobacter baumannii* were subjected to Imipenem isolates were subjected to a two different phenotypic tests; MBL screening was done by imipenem- EDTA double disc synergy test, imipenem- EDTA combined disc test.

Result:

Out of 91 *Acinetobacter baumannii* isolates,80 were imipenem resistant .MBL positive isolates were detected by DDST 60(65.9%) , and CDT 27 (29 .7%).

Conclusion: DDST is more effective than CDT to detect MBL producing isolates.

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INTRODUCTION

Acinetobacter baumannii are gram-negative coccobacilli that grow aerobically on standard laboratory media at an optimal temperature of 33-37°C. **{1}** It is an opportunistic pathogen that possesses a wide spectrum of antibiotic resistance in addition to being Gram-negative, oxidase-negative, non-fermentative, and non-motile coccobacilli. The bacteria is widespread, particularly in hospitals and other healthcare facilities.**{2}** The genus Acinetobacter, which is derived from the family Moraxellaceae , has at least 21 identified species, the most significant of which being Acinetobacter baumannii (A. baumannii), which is responsible for human infections.**{3}**

Acinetobacter baumannii, often known as A. baumannii, is a human pathogen that has gained significant attention due to its ability to cause several diseases, such as urinary tract infections, meningitis, pneumonia, and septicemia**.{4}** An infection of the bloodstream, central nervous system, skin, soft tissues, surgical sites, and bones have been documented most recently Over the past 20 years, substantial progress has been achieved in our understanding of this multidrug resistant bacteria. where it contributes to the development of dangerous infections include bloodstream infections, wound infections, pneumonia linked to ventilator use, and umeningitis. It mostly affects those with significantly weakened immune systems and is usually determined by previous antimicrobial therapy. A. baumannii is not a typical environmental organism, it is rarely discovered on human skin, and its natural reservoir is unknown .**{5}** Due to the low permeability of its outer cell membrane and the constitutive expression of certain efflux pumps, A. baumannii is intrinsically resistant to many antibiotics and disinfectants. In hospital settings linked to high antibiotic consumption, it can also accumulate components of resistance mechanisms encoded on plasmids, transposons, and integrons.**{6}**

Acinetobacter is generating a high rate of morbidity and mortality, particularly in intensive care units throughout many nations, and has developed resistance to the majority of effective antimicrobial drugs. Treatment of Acinetobacter infections is severely hampered by the advent of isolates that are extensively drug-resistant (XDR), pan drug-resistant (PDR), and multidrug-resistant (MDR). When treating illnesses brought on by gram-negative bacteria that are resistant to other β-lactam antibiotics, carbapenems are thought to be the most effective medication. Numerous investigations have revealed that these infections from various nations have significant rates of carbapenem resistance .**{7}** This multidrug-resistant and pan-resistant A.baumannii poses a challenge to the current antibiotic era.**{8}**

Multidrug-resistant bacteria can quickly spread in healthcare settings due to direct contact and other causes. These bacteria pose a significant epidemiological danger, particularly in healthcare-associated infections. They can develop resistance to carbapenems, a medicine used as a last option against multidrug-resistant pathogens.**{9}**

In the recent decade, Carbapenem-resistant Acinetobacter Spp. has become a major concern in the medical world. The ability to produce carbapenemase enzymes, such as metallo-beta lactamase (MBL), is the most common carbapenem resistance mechanism. The class B beta-lactamase (MBL), which hydrolyzes carbapenem and other beta-lactams except monobactam, can cause resistance to this antibiotic class. In A. baumannii, carbapenem resistance is mediated primarily by oxacillinases (oxas) and less frequently by metalloß-lactamases (MBLs).**{10}** MBL synthesis has been linked with resistance to aminoglycosides and fluoroquinolones, which limits therapy options. **{11}** A baumannii strains develop resistance to nearly all antimicrobial drugs, including carbapenems. The most common carbapenem resistance mechanism in this species is beta lactamase-mediated. Carbapenem resistance is primarily caused by the formation of carbapenemases of class A (KPC)3, class B (metallo-beta-lactamases: metallo-β-lactamases (MBLs) of IMP, VIM or SIM family) or class C carbapenem-hydrolyzing class D carbapenemases (CHDL)9. **{12}** In vitro, MBLs successfully hydrolyze all β-lactams except Aztreonam. A baumannii has been reported with three groups of MBLs (IMP, VIM, and SIM) and three groups of CHDLs (OXA group).**{13}**

Carbapenems are frequently used as last resort antibiotics in treating significant nosocomial infections caused by multidrug resistant bacteria. Resistance to carbapenem antibiotics due to carbapenem hydrolyzing enzymes is a global issue despite the fact that antibiotic resistance can be induced by various mechanisms.**{14}**

Carbapenems, which were once the principal treatment, are no longer effective in treating infections caused by this bacteria. To treat carbapenem-resistant A. baumannii infections, "last-line" antibiotics such as colistin, polymyxin B, or tigecycline are used. Sulbactam, a β-lactamase inhibitor, is effective against Acinetobacter species in vitro and can cure carbapenem-resistant bacteria.**{15}**

Several phenotypic methods for the detection of MBL producer isolates have been suggested by usingchelating agents never the less the specificity and sensitivity of this method are diverse. The combination disc assay (CD) and double disc synergism test (DDST) by use of Imipenem and EDTA as an MBL inhibitor are the most widespread phenotypic methods which are used in

numerous Studies. molecular method PCR with different primers have been used for confirmation of phenotypic assay since, blaVIM and blaIMP are the most common MBL.**{16}**

This study is conducted to identify carbapenem-resistant *Acinetobacter baumannii* in order to achieve effective treatment for this bacteria with multiple resistance, which represents a threat to public health.

AIMS & OBJECTIVES OF THE STUDY

AIMS

To detect metallo-beta-lactamase in *Acinetobacter baumanii* by phenotypic methods.

OBJECTIVES

1. Isolation and identification of *Acinetobacter baumannii* from various clinical samples.
2. To know the antibiotic susceptibility pattern of *A. baumanii* isolates
3. Comparison of phenotypic tests for the detection of MBL in *A. baumanii* isolates

REVIEW OF LITERATURE

History of genus A. baumannii

In a study conducted by **Howard, Aoife et al ,** By employing minimum media enhanced with calcium acetate, the Dutch microbiologist Beijerinck initially isolated the organism from soil in 1911. Around 43 years after it was first identified as Micrococcus calco-aceticus, Brisou and prevot suggested the name Acinetobacter (derived from the Greek word "akinetos," which means non-motile) to distinguish it from the motile species that belong to the genus Achromo bacter. When a thorough analysis of a number of organisms, including Micrococcus calco-aceticus, Alcaligenes hemolysans, Mima polymorpha, Moraxella lwoffi, Herellea vaginicola, and bacterium anitratum, was published in 1968, it became widely acknowledged that the genus Acinetobacter was the only one that included these species and could not be further divided into distinct species based only on phenotypic traits. Gram-negative, strictly aerobic, non-fermenting, non-fastidious, non-motile, catalase-positive, oxidase-negative bacteria with a DNA G + C concentration of 39% to 47% are included in the currently classified genus Acinetobacter. There are currently 26 recognized species and nine genomic species in the Acinetobacter genus, as a result of DNA-DNA hybridization research carried out by Bouvet and Grimnot in 1986. the A. calcoaceticus-complex is the term used to describe a group of four Acinetobacter species (A. calcoaceticus, A. baumannii, Acinetobacter genomic species 3, and Acinetobacter genomic species 13TU) that share so many morphological characteristics that it is difficult to distinguish between them. Since A. calcoaceticus, an environmental species, has not been linked to any clinical illnesses, its name may be deceptive the other three species in the A. calcoaceticus complex, however, may be the most clinically significant species because they are linked to nosocomial and community-acquired infections**.{17}**

Colony characteristics of A. baumannii

According to study by **Saad B. Almasaudi et al.,** Acinetobacter spp. are short, plump, and measure approximately 1.0–1.5 lm by 1.5–2.5 lm when measured during their rapid phase of growth. However, during their stationary phase, they frequently develop into more coccoid, and they are typically found in pairs or lengthy chains of varying length. Acinetobacter species are easily cultivated on standard laboratory media and are not fussy eaters. On blood agar plates, colonies have typical sizes and shapes: they are milky, 1-2 mm in diameter, smooth or mucoid (when a capsule is present), colorless (white or cream colored), and they incubate for 18–24 hours

at 37 °C. On eosin methylene blue agar, colonies are bluish to bluish gray in color. The colonies are light lavender in hue on Herellea agar and dark purple on Leeds Acinetobacter medium. The colonies have a pink color on a purple backdrop. Colonies grown on MacConkey agar are distinguished by their light lavender tint, which is indicative of non -lactose ferments. Strictly aerobic, non-motile, catalase-positive, indole negative, oxidase-negative, Gram-negative, citrate-positive, and with a G+ C content of 39–47% are the species that make up the genus Acinetobacter. These are enclosed coccobacilli rods that do not ferment glucose and are common in fluid media, especially in the early phases of growth. The ideal temperature is between 33 and 35 °C, and many strains are unable to convert nitrates to nitrites. Acinetobacter's cell wall is similar to that of Gram-negative bacteria, but destaining is challenging since it retains the crystal violet color, which may cause the bacteria to be mistakenly identified as Gram-positive cocci**.{18}**

Motility of A. baumannii

According to study by **Carole Ayoub Moubareck et al**, Due to its lack of flagella, A. baumannii has long been considered non-motile. Still, research indicates that this organism can use its twitching motility to survive infection and move over hospital surfaces. this type of motility uses type IV pili to propel cells in medium by extension and retraction movements. these pili are in charge of biofilm formation and gene transfer in addition to mobility. A model of Caenorhabditis elegans infection in A. baumannii demonstrated increased virulence because of motility. A. baumannii blood isolates were shown to be more motile the isolates of A. baumannii from sputum were seen to be more motile, which is likely indicative of a better survival advantage in blood. Apart from their twitching motility, certain isolates of A. baumannii exhibit surface-associated motility, which enables them to move on both living and non-living surfaces without the need for flagella. In addition, lipo-oligosaccharide synthesis, quorum sensing, type IV pili, and 1,3-diaminopropane—which provides the signals required for influencing surface-associated motility through quorum sensing—are required for this kind of motility**.{19}**

Biofilim of A. baumannii

In a study conducted by **Francesca Longo et al,** Studies on biotic and abiotic surfaces have shown that A. baumannii can adhere and produce biofilms. The ability of A. baumannii to adhere to epithelial cells is a general feature of this species, but it varies among different A. baumannii clinical isolates. This means that, when it comes to the interaction of A. baumannii with host cells, the first scanning electron microscopy (SEM) study showed that this adherence must be considered the foundation for colonization and subsequent host infection. Research has indicated that A.

baumannii clinical isolates have a strong inclination to develop biofilm on various substrata, including plastic and glass, when it comes to adherence on abiotic surfaces. Because it may form a biofilm on abiotic surfaces, A. baumannii plays a significant part in the development of nosocomial infections because it colonizes the surfaces of indwelling medical devices and hospital equipment, including urine catheters, central venous catheters (CVCs), endotracheal tubes. **{20}**

Clinical manifestation of A.baumannii

According to study by **Michael J. McConnell et al,** the most typical clinical manifestation of an A. baumannii infection is hospital-acquired pneumonia. patients in the intensive care unit who are on mechanical ventilation are most commonly affected by these infections. ventilator-associated pneumonia is believed to be caused by A. baumannii colonizing the airway by environmental exposure, which is followed by pneumonia developing. although the exact mortality rate directly related to A. baumannii infection has been disputed, reports of ventilator-associated pneumonia resulting from this pathogen range from 40% to 70%. However, a recent systematic review and a small number of studies have found that nosocomial A. *baumannii* infection is linked to higher attributable mortality. while far less common than nosocomial infection, community-acquired pneumonia due to A. baumannii has also been reported. between 40% and 60% of cases of community-acquired pneumonia result in death, and the illness is frequently linked to preexisting host characteristics like alcoholism or chronic obstructive lung disease. **{21}**

Classfication of beta-lactamase of A.baumannii

According to study by **Neetu Gupta et al ,** there are four kinds of beta-lactamase enzymes that are distinguished by their distinct hydrolytic processes and sequence motifs. Enzymes of classes A, B, and D hydrolyze carbapenems. Two kinds of carbapenemases are distinguished based on their active sites: (i) serine carbapenemases and (ii) metallo-b-lactamases (MBLs).

Carbopenemases of Class A

These enzymes' active sites include serine. β-lactamase inhibitors such as tazobactam and clavulanic acid have the ability to inactivate these serine-β-lactamases.

Carbopenemases of class B

These β-lactamases are MBLs with a catalytic site that contains zinc or another heavy metal. To activate and break the drug's beta-lactam ring, metallo beta-lactamase enzymes need both a zinc ion and a water molecule. In contrast to serine-β-lactamases, these enzymes are inhibited by clavulanate, sulbactam, and or tazobactam; instead, metal ion chelators such as dipicolinic acid, 1,10-o-phenanthroline, and ethylenediamine tetraacetic acid (EDTA) block them. MBL-type

carbapenemases have a 100–1000 times more hydrolytic activity than OXAs, while being less prevalent.

Carbapenemases of Class D

In Acinetobacter baumannii, carbapenem resistance is mostly caused by genes encoding oxacillinase enzymes (OXAs). These enzymes are serine-dependent; in vitro, sodium chloride (NaCl) may inhibit their activity at a concentration of 100 mM, but clavulanic acid, sulbactam, and tazobactam typically do not inhibit them.**{22}**

A.baumannii has the capacity to develop acquired resistance to a range of antibiotics used in therapy, according to study by **Sunil Kumar et al,** The amplification of resistance determinants has rendered a number of antimicrobial drugs, including penicillins, cephalosporins, tetracyclines, aminoglycosides, and quinolones, ineffective when used against A. baumannii therapy. The mechanism underlying this could involve the following: overexpression of efflux transporters, overexpression of antimicrobial modifying enzymes, modification of target sites, insertion of new resistance determinants, and genetic alteration leading to modification of the membrane fusion proteins Microorganisms 2021 (OMPs). Consequently, carbapenems are the only choice of treatment alternatives due to their enhanced activity and reduced toxicity for A. baumannii infections, In the past few decades, A. baumannii caused an array of nosocomial infections, which were successfully treated with gentamicin, minocycline, nalidixic acid, ampicillin, or carbenicillin, either as monotherapy or in combinations, but global surveys have shown an increased resistance in the hospital isolates Several mechanisms of resistance have been implicated in the augmentation of resistance in A. baumannii:

1. Enzymatic mechanisms including; deferent _-lactamases.
2. Non-enzymatic mechanisms involving efflux pumps and membrane permeability.
3. Change in the sequence of penicillin-binding proteins (PBPs).

The first two of these are especially important in transferring resistance to A. baumannii clinical isolates. Antibiotic resistance to penicillins, cephalosporins, and carbapenems increased as a result of bacterial _-lactamase activit . Lactamases are produced by A. baumannii and are encoded by a variety of chromosomal or plasmid genes**.{23}**

High levels of resistance to widely used antibiotics were seen in A. baumannii isolates, according to study by **Mubashir Ahmad Khan et al,** The most successful treatment for it was amikacin.**{1}**

Multiple drug resistance is emerging as a global medical concern, according to a study by **Sakar B. Smile et al,** using Acinetobacter baumannii. The rapid development of newer beta lactamases, which cause treatment failure, is cause for concern. Treatment for Acinetobacter baumannii infections with carbapenems is well-established, and the use of colistin and polymyxins is limited due to the drug's resistance. To treat multidrug resistant Acinetobacter baumannii infections, several novel medications are still being researched and combined drug therapy is being used in hospitals.**{2}**

The study conducted by **Mariya Rouf1, et al,** According to our research, the Imipenem-EDTA combination disc test is the most effective test for detecting the synthesis of carbapenemase, closely followed by tests like MHT. These tests can be used frequently to determine whether or not carbapenemase manufacturers are present because they are simple to do and reasonably priced**.{3}** The study conducted by **Taghrid Gamal El-Din et al,** Acinetobacter species exhibit resistance to numerous antibiotic classes. These pathogens' multidrug resistance is caused by their production of ESBLs and MBLs **{4}**

According to a study by **Azimi Z. et al,** the Double Disc Synergy Test (DDST) may be a more useful method for detecting MBL-producing A. baumannii than the combination disc.**{5}**The combined disc test, modified Hodge test, and E-test are all equally effective in detecting MBL, according to a study done by **Shivaprasad et al,** E-test can be substituted with straightforward MHT and CDT. They are easy to use, reasonably priced, and can be included in lab experiments to monitor the emergence of MBLs in MDR A. baumannii. **{8}** According to study by **Leila Azimi, et al,** was suggests that Double Disc Synergy Test (DDST) may be more practical than the combination disc for the detection of MBL-producing A. baumannii**.{10}**

The study conducted by the **Dr. Dinesh Kumar Kalra et al ,** along with others When it came to identifying isolates that produced MBLs, the DET and CDT demonstrated great agreement with molecular techniques. They can be regularly utilized in clinical laboratories and are inexpensive and simple to perform**.{11}** According to this study by **Branka Bedenić1 et al ,** total of 172 *A. baumannii* strains (123 carbapenemase positive and 49 carbapenemase negative were analyzed. Phenotypic detection of MBLs was performed by the combined disk test with EDTA (CDT-EDTA) and EPI-dilution test (EPI-DT). Both tests were positive in all 11 isolate possessing VIM-1 MBL, showing 100% sensitivity. However, false positive results were observed in strains with class D carbapenemases using both tests, i.e. all OXA-23 and OXA-24/40 producing organisms and most

OXA-58 positive strains (77% with CDT-EDTA *vs*. 65% with EPI-DT). False positive results can occur because oxacillinases are converted to a less active state in the presence of EDTA, leading to augmentation of the inhibition zone around the carbapenem disk or reduction of carbapenem minimum inhibitory concentrations. This study showed high sensitivity but low specificity of phenotypic methods in the detection of MBLs **.{12}**

In a study conducted by **Muneeza Anwar et al,** Out of 112, 66 (58.9%) isolates were resistant to both imipenem and meropenem (OXOID). These resistant isolates were tested for carbapenemase production, and 55 (83.3%) were carbapenemase producers by Modified Hodge Test. These isolates were further tested for MBL production by combined disk test and double disk synergy test. Out of 66, 49 isolates were positive by both methods, CDT and DDST, and only one isolate was detected as negative (with kappa value = 0.038). All MBL producing strains showed remarkable resistance to cephalosporins, fluoroquinolones, aminoglycosides, and piperacillin/tazobactam (OXOID). The antibiotic resistance was very high in A. baumannii which were isolated from children in Pakistan specially attending a nephrology unit.**{14}**

METHODOLOGY

MATERIALS AND METHOD

Source Of Data: The study is carried out in the Department of Microbiology, Shri B .M Patil Medical Collage, Vijayapura, Karnataka, India

Type Of Study: Cross-sectional study

Study Subjects: Patients attending Shri B.M Patil Medical College and Hospital Vijayapura.

Study Period: One -year

The study protocol was approved by the Institutional Ethical Committee (BLDE(DU)IEC/715/2022-23) of the Shri B M Patil Medical College Hospital and Research Centre, Vijayapura.

Inclusion Criteria: Clinical samples showing a growth of *Acinetobacter baumanii* were included.

METHODS OF DATA COLLECTION: A total of 91 isolates were collected from patients attending Shri B .M Patil Medical College, Vijayapura, Karnataka, India. Written informed consent forms of study were collected from all participants of this study

METHODS

Sample collection and processing

A total of 91 isolates were collected from infected patients from inpatients and outpatients of BLDE Hospital, Vijayapura. Various samples were collected, like urine, swabs, blood, sputum, and other body fluids.

Specimen were collected according to universal precaution standard guidelines and were sent for processingas soon as possible. If the delay is, then samples were stored at a 2-8°C refrigerator.

Isolation identification of *A baumannii*:

All 80 *A baumannii* isolates were identified by a conventional method as per standard microbiology laboratory protocol and finally identified by observing the culture characteristics on routine laboratory culture media, namely Blood agar and McConkey agar plates. The bacterial colonies showing non-lactose fermenting pale color on MacConkey agar plates, Oxidase negative, Triple sugar Iron showing alkaline slant with no change in butt will be included in the study.

Antimicrobial susceptibility

Isolates yielding non-lactose fermenting colonies that are oxidase negative and TSI agar K/Nc were subjected to vitek for identification and ABST using card ASTN 406. Which contains drugs like piperacillin, ceftazidime, cefoperazone, cefepime, aztreonam, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, minocycline, tigecycline, Fosfomycin, colistin, trimethoprim.

Phenotypic characterization of *Acinetobacter baumannii*

phenotypic tests were done to detect MBL production in *Acinetobacter baumannii* strains which are resistant to imipenem.

Following are the two tests

1. Double Disc Synergy Test. (DDST)
2. Combined Disc Test. (CDT)

1. Imipenem -EDTA Double Disc Synergy Test (DDST)

The overnight broth cultures of test isolates along with standard control strains (opacity adjusted to 0.5 McFarland opacity standard) were inoculated onto Mueller- Hinton agar CLSI plates as lawn culture according to the recommendation after drying a 10 µg imipenem disc was placed on the lawn culture with a distance of 15mm center to center from a blank disc. 10 µl of 0.5 M EDTAwas added to the blank disc and incubated overnight. presence of an enlarged zone of inhibition towards the EDTA disc was interpreted as positive for MBL production.[8]

2. Imipenem -EDTA Combined Disc Test (CDT)

The test isolates, along with standard control strains (opacity adjusted to 0.5 Mc Farland opacitystandard), will be lawn cultured on the Mueller-Hinton agar plate as recommended by CLSI. After drying, two 10µg imipenem discs. were placed on the lawn culture with a 20mm

distance from center to center of the disc. 10µl of 0.5 M EDTA was added to one of the imipenem discs and incubating at overnight. isolates showing >7 mm increase in the inhibition zone size of imipenem -EDTA disc than the imipenem disc alone is considered as MBL producers.[8]

SAMPLE SIZE

With an anticipated Proportion of MBL 30% among isolated multidrug resistant (1), the studywould require a sample size of 80 isolates with a 95**%** level of confidence and 10% absoluteprecision**.**

(Referred: Statulator software ttp://statulator.com/SampleSize/ss1P.html)

Formula used

$$\frac{n=z^2\ p*q}{d2}$$

Where Z= Z statistic at α level of significance

d^2= Absolute error

P= Proportion

rateq= 100-p

STATISTICAL ANALYSIS

- The data obtained will be entered in a Microsoft Excel sheet, and statistical analysis willbe performed using JUMP Pro 16 software.
- Data will be presented as Mean ± SD, or Median and inter-quartile range Frequency, percentages and diagrams.

RESULTS

Isolation and identification of *A baumannii*

All 91 A baumannii isolates were identified by conventional method as per standard microbiology laboratory protocol and finally identified by observing the culture characterstics on routine laboratory culture media namely Blood agar and Macconkey agar plates. The bacterial colonies showing non-lactose fermenting pale color on MacConkey agar plates, Oxidase negative, Triple sugar Iron showing alkaline slant with no change in butt will be included in the study.

FIG NO 1 : MacConkey agar plate showing a non-lactose fermenting pale color

FIG NO 2 : Blood agar plate showing a translucent to opaque, convex, smooth surface, entire margin

Acinetobacter spp. growth on 1 MacConkey's agar 2. Blood agar. and Non-lactose fermenter colonies on Macconkey agar. Translucent to opaque, convex, smooth surface, entire margins and 0.5-2.0 mm in diameter on blood agar .

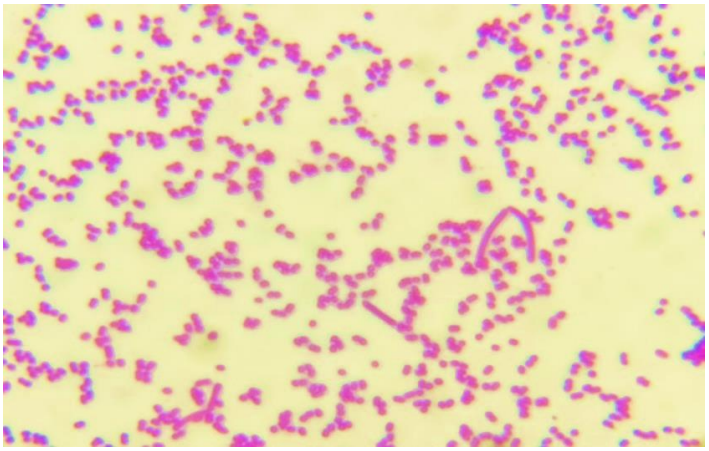

Fig no 3: Microscopic observation of Acinetobacter spp. Gram negative cocco bacilli (1000X

Out of 91 clinical samples ,80 imepenem resistance of Acinetobacter baumannii were found. The data showed that the majority of A. baumannii was found from pus followed by ET tube, Blood sputum, urine, csf.

Table No : 1. Distribution of isolates according to specimen types

Specimen Type	No. of patients	Percentage
PUS	43	47.3
ET TUBE	24	26.4
BLOOD	14	15.4
SPUTUM	6	6.6
URINE	3	3.3
CSF	1	1.1
Total	91	100.0

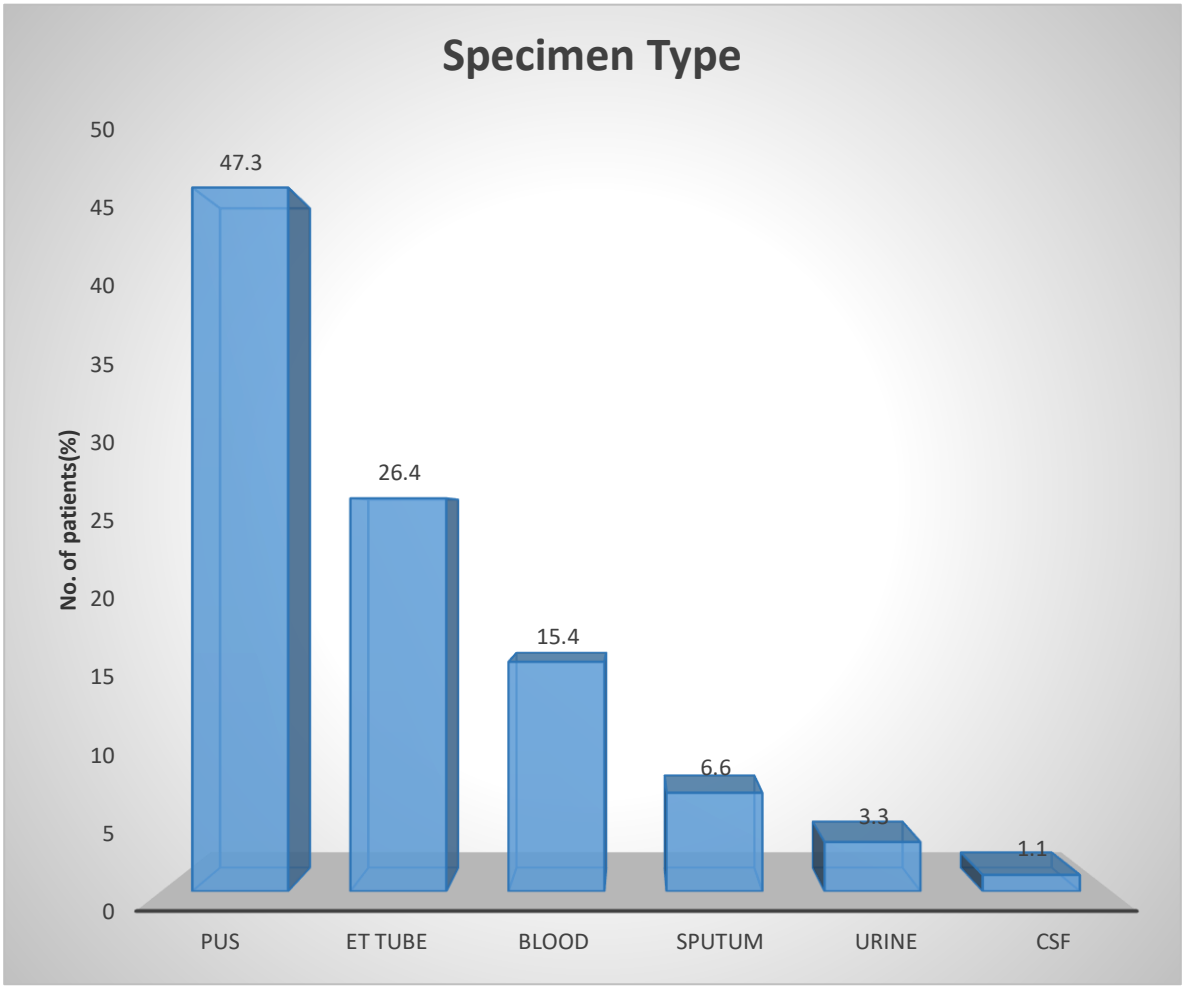

Fig no : 4 Distribution of Acinetobacter infection amoung from various clinical specimens.

These isolates were isolated from various clinical specimens with high array of Pus 43(47.3 %), followed by ET Tube 24 (26.4%) and Blood 14 (15.4 %), Sputum 6 (6.6%), Urine 3 (3.3%) with low array CSF 1 (1.1 %)

Table No: 2 Distribution of patients according to gender

Gender	No.of patients	Percentage
MALE	68	74.7
FEMALE	23	25.3
Total	91	100.0

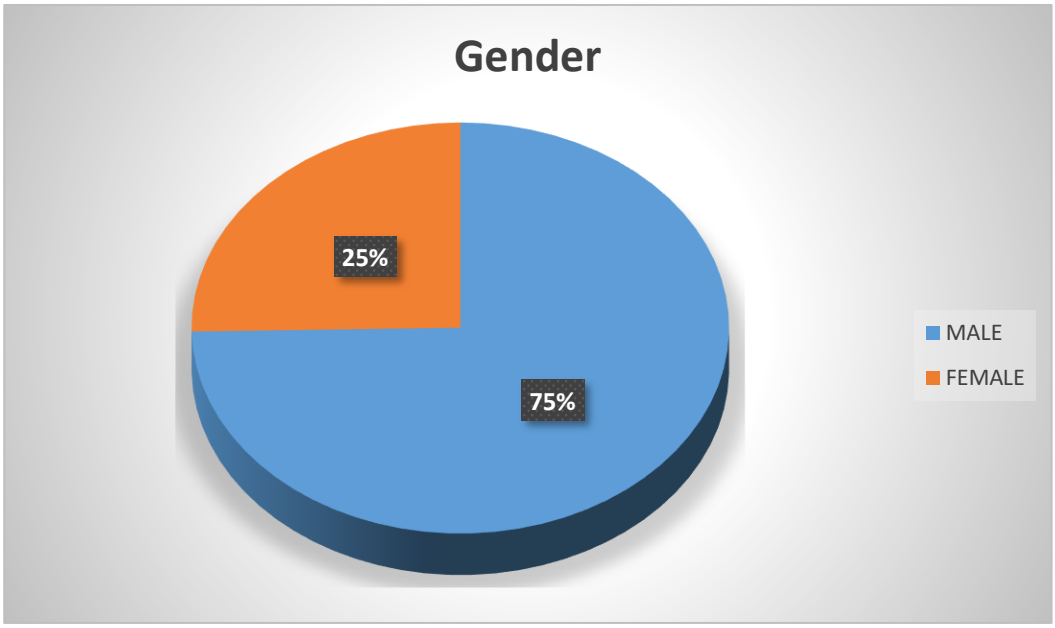

Fig no : 5 Distribution of isolates according to gender

According to this report , The males were found to be more predominant 63(74.7 %) than female patients 23(25.3%) affected by these bacterial infection of acientobacter baumannii

Antimicrobial susceptibility test

All clinical isolates of Acinetobacter identified from NFGNB were subjected to Antibiotic susceptibility test by vitek for identification and ABST using card ASTN 406. according to the CLSI standards and the antibiogram patterns were recorded.

Fig no : 6 vitek 2 compact

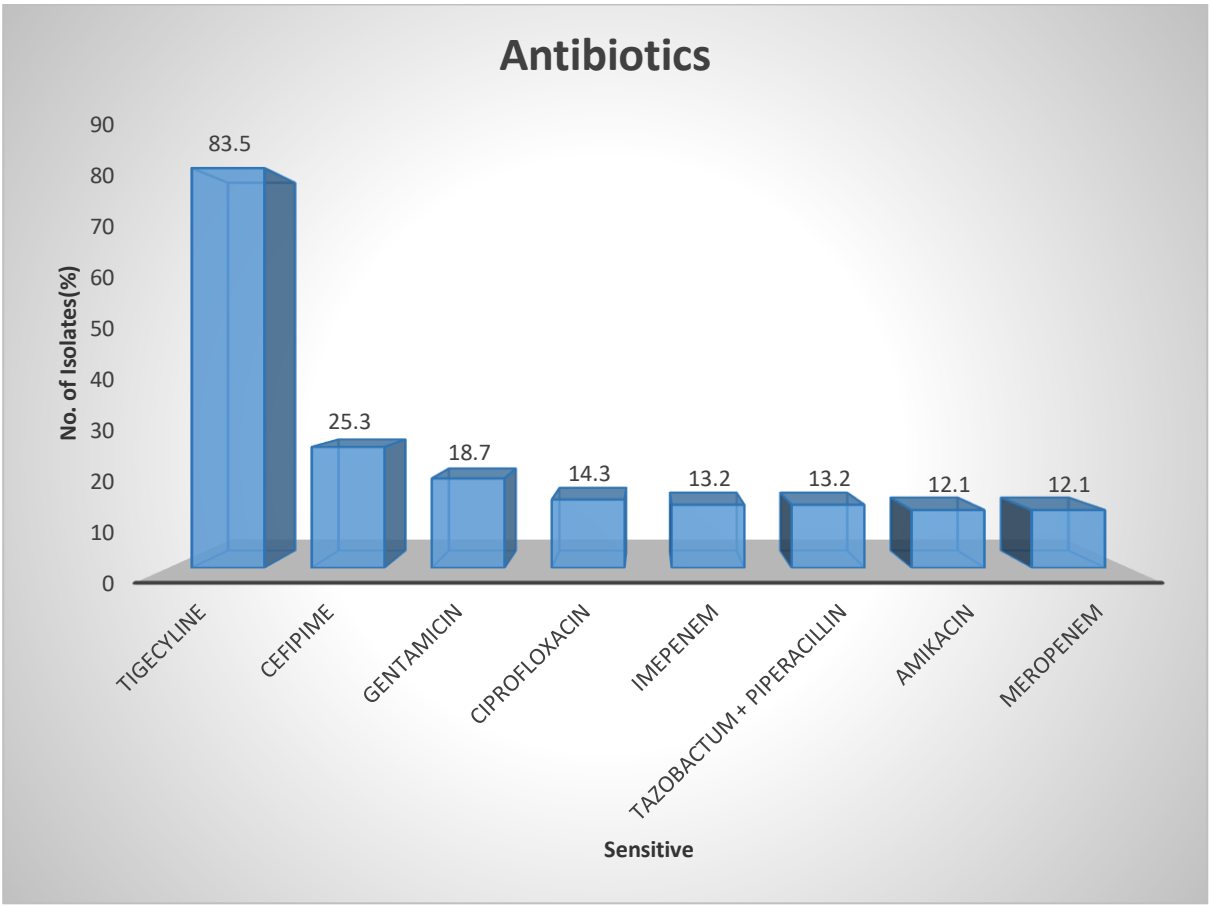

Fig no : 7 Antibiotic susceptibility patterns for Acinetobacter baumannii

Fig no ; 7 shows susceptibility to the following :Tigecyline 76(83.5%) followed by cefipime 23(25.3%) , Gentamicin 17(18.7%) ,and Ciprofloxacin 13(14.3%), Tazobactum +piperacillin 12(13.2%), Imipenem 12(13.2%), Meropenem 11(12.1%), Amikacin 11(12.1%)

PHENOTYPIC TESTS FOR MBL PRODUCTION OF ACIENTOBACTER BAUMANNII

S.NO	Phenotypic test	Positive		Negative	
		No. of Isolates	Percentage	No. of Isolates	Percentage
1	**DOUBLE DISC SYNERGY TEST**	60	65.9	31	34.1
2	**COMBINED DISC TEST**	27	29.7	64	70.3
3	Total	91	100.0	91	100.0

Table no : 3 Comparative evaluation of MBL production by phenotypic tests

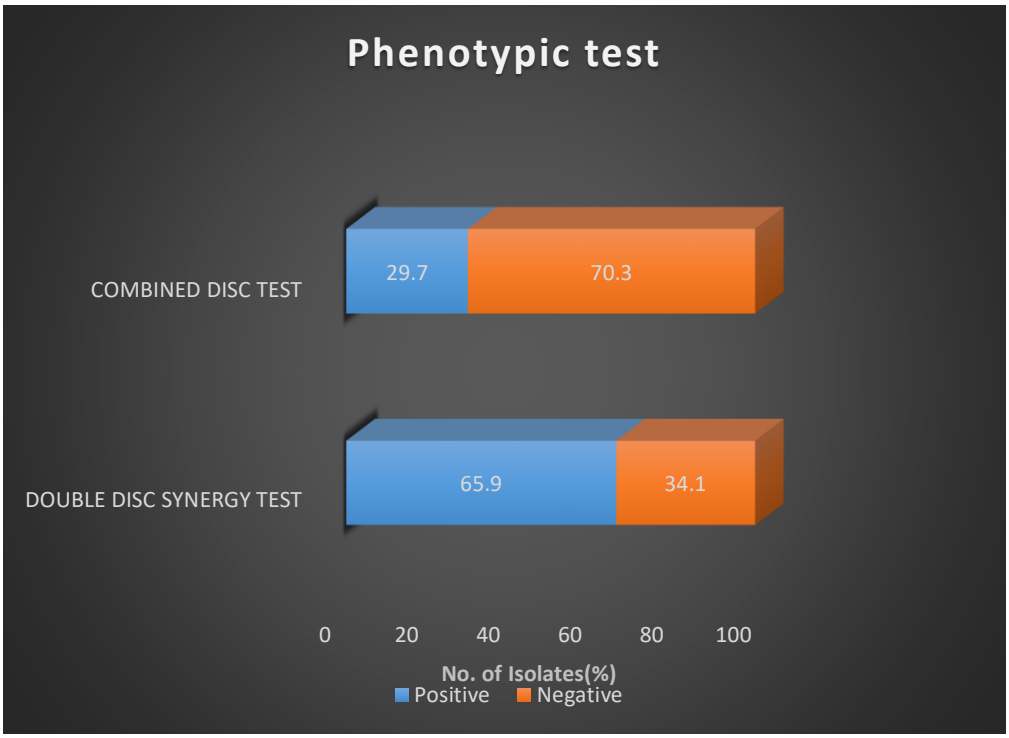

Fig no : 7 phenotypic detection by Double disc synergy test and combined disc test

PHENOTYPIC DETECTION OF MBL PROUDUCTION IN ACIENTOBACTER BAUMANNII

1. **Double disc synergy test :** The zone should be 15mm from centre to centre (imipenem blank disc) was interpreted as MBL Positive according to the CLSI gudilines in this test 60(65.9%) are positive and Negative is 31(34.1%) .

2. **Combined disc test :** The increase of inhibition zone >7mm of imipenem EDTA disc than the imipenem disc alone interpreted as MBL Positive according to the CLSI guidelines in this test 27 (29 .7%) are positive and Negative is 64 (70.3%).

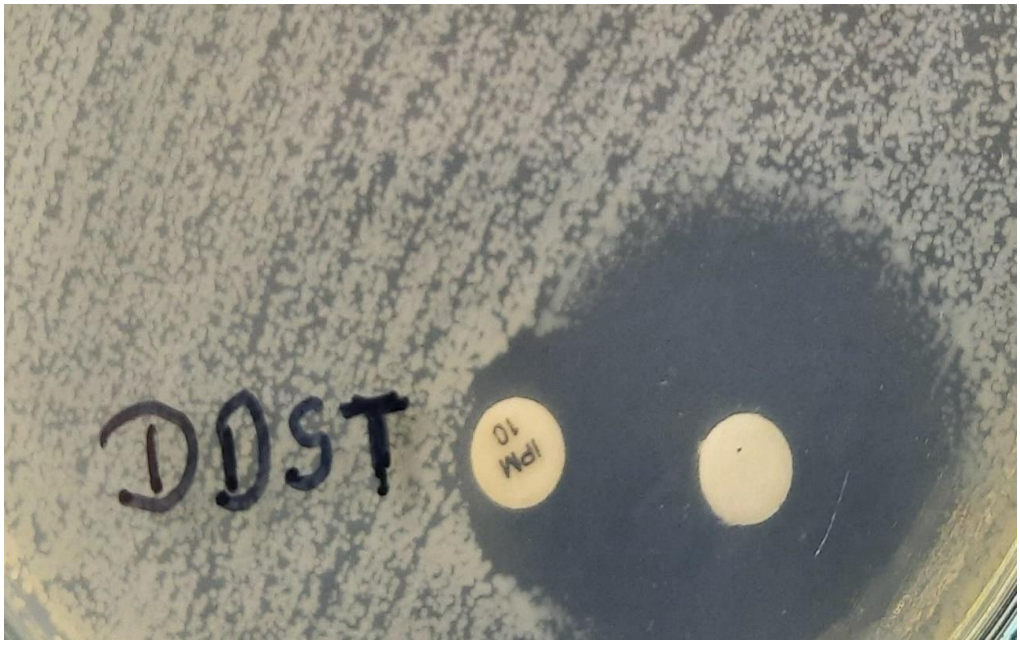

Fig no : 8 phenotypic detection by Double disc synergy test

The zone should be 15mm from centre to centre (imipenem blank disc) .

Fig no :9 showing the imipenem - EDTA Combined disc Test :

The increase of inhibition zone >7mm of imipenem EDTA disc than the imipenem.

DISCUSSION

A .baumannii infections present a global medical challenge. The interest in this organism has been growing rapidly because of the emergence of multi-drug resistant strains (MDR), some of which are pan resistant to antimicrobial agents.**{24}**

ß lactam antibiotics such as carbapenems are one of the potent drugs to treat infection by MDR. Prevalence of carbapenemase among gram negative bacilli varies greatly from country to country and among different institutions within the country**.{25}**

In this study conducted by Muneeza Anwar et al **,**The most frequent site of infection was urinary tract infections (21.2%) followed by blood stream infections (18.2%) and respiratory infections (13.6%). The results were different from other studies which reported that respiratory tract infections were the common site of infection**.{14}** Noori et al. found the highest percentage of MBL producing A. baumannii in respiratory tract specimens (52.8%) followed by urine (26.9%) and blood (7.4%). **{26}** Another study was conducted by de Carvalho et al. which described that the most frequent site of isolation was tracheal secretion (56.3%), followed by the catheter tip (16.9%), blood (7%), and urine (7%). **{27}** In the present study, A. baumannii were isolated from various clinical specimens like Pus 43(47.3 %), followed by ET Tube 24 (26.4%) ,Blood 14 (15.4 %), Sputum 6 (6.6%), Urine 3 (3.3%) and CSF 1 (1.1 %). **Table no :1** shows the specimens types.

According to the study by Begum et al. performed at Quaid-i-Azam, University Islamabad, 42.85% of MDR *A. baumannii***.** isolates were isolated from male patients and 58.5% from female patients. There were 74.2% male and 25.8% female patients. **{28}**A study by Alm-El-Din et al. reported more males (51.5%) than females (48.8%) **{29}.** Islahi . (2014) also observed a high percentage of more isolates seen in males (76.0%) than females (23.9%) **{30}**Similar results were seen in a study performed by Peymani et al. in which 72% were male and 28% were female patients. **{31} i**n the present study more isolates were obtained from male patients 68(74.7%) compared to female patients 23(25.3%).**Table no: 2** shows the isolates gender.

In a study conducted by Enas A. Daef et al , 31.4% of all A. baumanni isolates were imipenem resistant. For other antibiotics, we recorded high rates of resistance to ciprofloxacin (64.7%), amoxicillin clavulanic acid (58.8%), amikacin (58.8%) and ceftriaxone (56.9%).**{24}** The least rates of resistance was against tetracycline (25.5%). Our results were lower than that reported in another Egyptian study where resistance rates approached nearly 100% against many antibiotics among carbapenem resistant Acinetobacter isolates**.** In the present study the following Antibiotic susceptibility patterns for Acinetobacter baumannii **[fig no ; 7]** shows susceptibility

to the following :Tigecyline 76(83.5%) followed by cefipime 23(25.3%) , Gentamicin 17(18.7%) ,and Ciprofloxacin 13(14.3%), Tazobactum +piperacillin 12(13.2%), Imipenem 12(13.2%), Meropenem 11(12.1%), Amikacin 11(12.1%).

Simple and accurate test are needed to detect mbl producers there are standard guideline available for detection of mbl .different studies have reported the use of various methods .most of the studies have used Imipenem -EDTA double disc test Imipenem -EDTA combined disc test .**{8}**

In this study conducted by the Enas A. Daef et al, 86.3% of A. baumannii isolates produced MBL S by the DDST. **{24 }**The percentages varied in many studies from 14% to 70.9% Lee et al., ; Uma et al; Anwar, Amin et al , We reported the sensitivity of the DDST to be 86.2% and 100% for IMP sensitive and resistant strains respectively**.{32,33,34}** Our results were some what higher than those of Franklin et al. who reported a sensitivity of 79%and a specificity of 98% **.{ 35 }**Other studies documented a higher sensitivity of DDST (100%) as was reported by Yan et al. **{ 36}** Lee et al. reported it to be 33.3%. The variation in different studies may be due to the different gold standard taken to which the phenotypic test is compared to in each study**.{32}** DDST results are more subjective as it depends upon the technician's expertise to discriminate true synergism from the intersection of inhibition zones Pandya et al 2003**.{37}** The studies differed in the cut off chosen for MBL detection. Our cut off value was 4 mm according to Franklin et al.while it was 7mm **{35}**for others Renu et al., We reported the sensitivity of the DDST to be 86.2% and 100% for IMP sensitive and resistant strains respectively**. {38}**

Several studies have reported the use of DDST as one of the convenient methods for the detection of Ambler class B MBL production and the positivity has varied from 14.8%- -72%. **{39}** According to their finidings, DDST was found to be more reliable and reproducible with high rate of positivity. similarly, in our study DDST showed the highest positivity.sDDST was found to be superior to CDT and shown **[fig no : 7]** DDST in this test 60(65.9%) are positive and Negative is 31(34.1%).and CDT in this test 27 (29 .7%) are positive and Negative is 64 (70.3%)

s.no	Authors	No of isolates	DOUBLE DISC SYNRGY TEST	COMBINED DISC TEST
1	Lee K, et al.,2003 [40]	73	45(61.64%)	-
2	Behera B, et al., 2008 [41]	63	36(57.14%)	48(76.19%)
3	Jesudasan M,et al 2005[42]	50	36(72%)	-
4	John S et al.,2011[43]	242	36(14.8%)	-
5	Shivaprasad et al [8]	168	57(67.05%)	69(81.18%)
6	Dr Richa. Agrawal et al (2015) [44]	120	45(80.3%)	47(84%)
7	Kareem. A. Sofy et al,[45]	150	32(65.3%)	35(71.4%)

8	S Vinoba, et al, [46]	50	15(25.4%)	29(49.2%)
9	Present study	80	60(65.9%)	27 (29 .7%)

Table no 5 : Comparative studies by other authors

CONCLUSION

In the collection samples total no of 91various clinical samples were obtained from Out of 80 imepenem resistance of Acinetobacter baumannii were found. The data showed that the majority of A. baumannii was found from Pus 43(47.3 %), followed by ET Tube 24 (26.4%) and Blood 14 (15.4 %), Sputum 6 (6.6%), Urine 3 (3.3%) with CSF 1 (1.1 %) .

According to this report ,The males were found to be more predominant 63(74.7 %) than female patients 23(25.3%) affected by these bacterial infection of acientobacter baumannii.

Antibiotic susceptibility patterns for Acinetobacter baumannii shows susceptibility to the following :Tigecyline 76(83.5%) followed by cefipime 23(25.3%) , Gentamicin 17(18.7%) ,and Ciprofloxacin 13(14.3%), Tazobactum +piperacillin 12(13.2%), Imipenem 12(13.2%), Meropenem 11(12.1%), Amikacin 11(12.1%).

in our study DDST showed the highest positivity.s DDST was found to be superior to CDT and DDST in this test 60(65.9%) are positive and Negative is 31(34.1%).and CDT in this test 27 (29 .7%) are positive and Negative is 64 (70.3%)

Comparasion of phenotypic methods as mentioned above for MBL production in bacteria indicates that the DDST provides the highest rate of positive test.

Growing multidrug resistance (MDR)and resistance to highly strong antimicrobial drugs pose therapeutic challenge in our hospitals and healthcare settings, To stop multidrug resistant organisims from spreading throughout healthcare institutions, adequate infection prevention and control measures as well as ongoing surveillance must be reinforced.

Addressing the problem of the high incidence and prevalence of multi- drug resistant Acinetobacter infection- which represents a therapeutic impasses- necessitates both increasing hand cleanliness and antibiotic use in the hospital as well as regulating the hospital environment.

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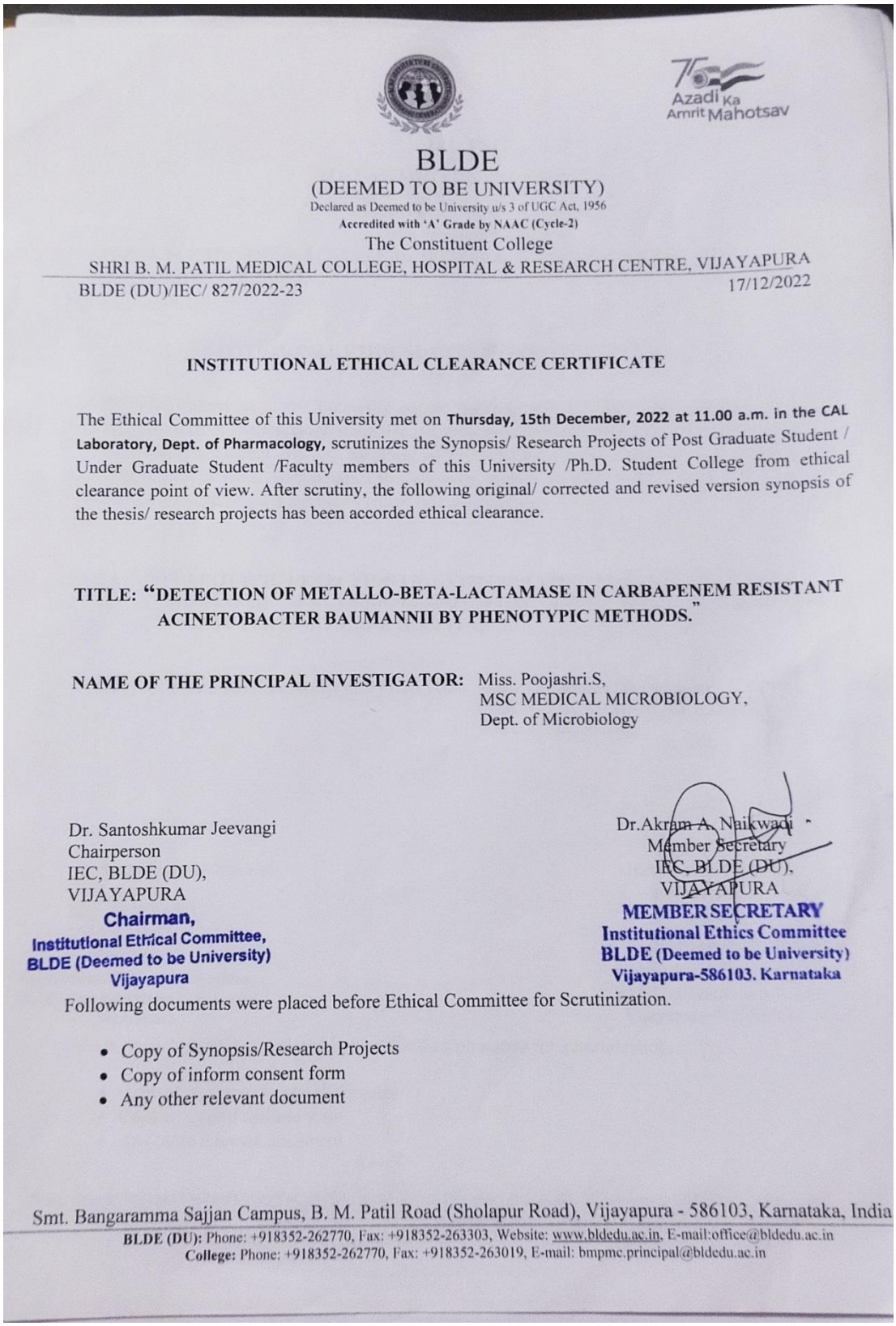
ANNEXURE – I

INSTITUTIONAL ETHICAL CERTIFICATE



BLDE
(DEEMED TO BE UNIVERSITY)
Declared as Deemed to be University u/s 3 of UGC Act, 1956
Accredited with 'A' Grade by NAAC (Cycle-2)
The Constituent College
SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/ 827/2022-23 17/12/2022

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on **Thursday, 15th December, 2022 at 11.00 a.m. in the CAL Laboratory, Dept. of Pharmacology,** scrutinizes the Synopsis/ Research Projects of Post Graduate Student / Under Graduate Student /Faculty members of this University /Ph.D. Student College from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

TITLE: "DETECTION OF METALLO-BETA-LACTAMASE IN CARBAPENEM RESISTANT ACINETOBACTER BAUMANNII BY PHENOTYPIC METHODS."

NAME OF THE PRINCIPAL INVESTIGATOR: Miss. Poojashri.S,
MSC MEDICAL MICROBIOLOGY,
Dept. of Microbiology

Dr. Santoshkumar Jeevangi
Chairperson
IEC, BLDE (DU),
VIJAYAPURA
Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)
Vijayapura

Dr.Akram A. Naikwadi
Member Secretary
IEC, BLDE (DU),
VIJAYAPURA
MEMBER SECRETARY
Institutional Ethics Committee
BLDE (Deemed to be University)
Vijayapura-586103, Karnataka

Following documents were placed before Ethical Committee for Scrutinization.

- Copy of Synopsis/Research Projects
- Copy of inform consent form
- Any other relevant document

Smt. Bangaramma Sajjan Campus, B. M. Patil Road (Sholapur Road), Vijayapura - 586103, Karnataka, India
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College: Phone: +918352-262770, Fax: +918352-263019, E-mail: bmpmc.principal@bldedu.ac.in

ANNEXURE – II

SCHEME OF CASE TAKING:

PATIENT PROFORMA

Patients Details

Name :

Age :

Sex :

Occupation :

Residence :

Contact No :

OPD/IP NO :

Lab No :

Other clinical history:

Clinical history:

Previous Treatment history:

- History of any previous visit or admission.
- Earlier medicationhistory or any current medications.
- If the patient is involved in any surgery.
- If the patient has a history of long stays in the hospital.

Laboratory Diagnosis:

From all Clinical isolates, we obtain *Acintobacter baumanii* andobserve ABST by standard Antibiotic disks used are as follows.

ANNEXURE – III

INFORMED CONSENT FORM

GUIDE: DR. JYOTHI P MD

PG STUDENT: POOJASHREE S [MSC MEDICAL MICROBIOLOGY]

Purpose Of Research:

I have been informed that this study is bacteriological based and for studying the antibiogram of the study organism. This study was carried out in a tertiary care center in BLDE Hospital Vijayapura. I have been given free choice for participation in this study. The study will help in giving appropriate treatment to the patient, and this will enhance better patient management.

PROCEDURE: I understand that I will undergo a detailed history, after which necessary investigations will be done.

Risk And Discomforts:

I understand that I may experience some discomfort during the sampling procedure. The procedures of this study are not expected to exaggerate those feelings which are associated with the usual course of study.

Benefits:

I understand that my participation in the study as one of the study subjects will help the researcherto identify antibiotic resistance and the prevalence of serotype. The study will have more indirect benefits to me than the potential benefits of the study for choosing appropriate antibiotic management.

I have explained to Mr./Mrs._____the purpose of the research, the procedures required and the procedures required possible risk factors to the best of my ability.

_____

Miss. POOJASHREE S DATE:

(investigator)