T.C.
TOKAT-GAZİOSMANPAŞA ÜNİVERSİTESİ
Graduate Education Institute
Department of Medical Microbiology
Master's Program with Thesis

Investigation of carbapenemase production in carbapenem resistant *Acinetobacter baumannii* isolates by phenotypic and genotypic methods

Master Thesis

Prepared By
Daban Kanabi Rashid

Supervisor
DOÇ. Dr. Umut Safiye ŞAY COŞKUN

TOKAT – 2022

ETHICS CONTRACT

T.C.
TOKAT GAZİOSMANPAŞA UNIVERSITY
TO THE INSTITUTE OF GRADUATE STUDIES DIRECTORATE

With this document, I declare that the Master's thesis named "Investigation of carbapenemase production in carbapenem resistant *Acinetobacter baumannii* ısolates by phenotypic and genotypic methods" is an original work which I prepared in accordance with the guidelines of Tokat Gaziosmanpaşa University - Institute of Graduate Studies and under the consultancy of DOÇ. Dr. Umut Safiye ŞAY COŞKUN. And i hereby declare that all the information in the current thesis have been gathered and presented in line with academic rules and ethical principles, and as a prerequisite for these rules and principles, I have cited all data, thoughts, and results that do not belong to me.

Tokat Gaziosmanpaşa University Ethics Committee for Clinical Research decision number: 21-KAEK-242
Tokat Gaziosmanpaşa University Scientific Research Center project number: 2021/19

(22/08/2022)
The Student Who Prepared the Thesis
DABAN KANABI RASHID

JURY ACCEPTANCE AND APPROVAL

The defense examination of the thesis study titled “Investigation of carbapenemase production in carbapenem resistant *Acinetobacter baumannii* isolates by phenotypic and genotypic methods’’, which was prepared by **DABAN KANABI RASHID** was held on 22/08/2022. It was accepted by the jury members of that session as a Master Thesis at Tokat Gaziosmanpaşa University, Institute of Graduate Studies, Department of Medıcal Microbiology.

Jury Members (Title, Name Surname)	Signature
Chairman : Prof.Dr. Yunus Bulut	
Member : Doç.Dr. Emel Çalışkan	
Member : Doç.Dr. Umut Safiye Şay Coşkun	

CONFIRMATION

22/08/2022

Prof. Dr. Mehmet GÜNEŞ
Director of institute of graduate studies

ACKNOWLEDGMENTS AND FOREWORD

Thanks be to god for giving us peace, success and for all the opportunities w have had to light our path.

I would like to express our thanks to our supervisor **(DOÇ. Dr. Umut Safiye ŞAY COŞKUN),** Who assisted us with this project.

I would like to offer our thanks to the our head of department **(Prof. Dr. Yunus BULUT)** and all academic staffs in the college and Hospital for their efforts with us**.**

I would like to thank each of **Dr. Nihan BOZKURT**, **Dr. Yelda**, **Kubra ŞAHIN** and **Sadegül ŞAVKIN** for their great help.

ABSTRACT

Investigation of carbapenemase production in carbapenem resistant *Acinetobacter baumannii* isolates by phenotypic and genotypic methods

DABAN KANABI RASHID

Master's program, Tokat Gaziosmanpaşa University, Institute of Health and Science, Department of Medical Microbiology

Thesis supervisor: **DOÇ. Dr. Umut Safiye ŞAY COŞKUN**

The nosocomial infection carbapenem-resistant *Acinetobacter baumannii* has attracted worldwide attention as a serious threat. Especially pneumonia and bloodstream infections, both of which have a high mortality risk, are the most common symptoms among the most vulnerable individuals. There has been not enough improvement the new antibiotic agent despite the constant attempts to improve medical treatment techniques. There are just a few antibiotics that can be used to treat carbapenem-resistant *A. baumannii* infections because of a significant resistance profile.

The study included 80 carbapenem-resistant *A. baumannii* isolated from (pus, wound swab, catheter, sputum, and urine) samples taken from different clinics in Tokat Gazi Osmanpaşa university hospital. Identification and antibiotic susceptibility test of the isolates were done by the Vitek-2 (bioMerieux, France) automated system . The disk diffusion method was used to determine resistance to carbapenems too. The carbapenem-inactivation method was used to illustrate carbapenemase enzyme and polymerase chain reaction to identify carbapenem resistant genes.

The highest percentage of carbapenems-resistant strains have been found in sputum specimens and neurosurgery clinics. The carbapenemase production in isolates, (n = 50, 63%) of the isolates were found to be positive by the carbapenem-inactivation method. In this investigation, carbapenem-resistant genes were 14/38 (33%) of the isolates were determined to be positive for IMP, 11(14%) isolates were detected positive for blaOXA-48, for NDM, 9(11%) isolates were determined as positive, and 8(10%) isolates were positive for NDM+KPC and KPC weren't detected without NDM.

In contrast to the phenotypic detection of carbapenemase production in isolates, 50/80 (63%) of the isolates were positive by PCR. In this study, despite all isolates being resistant to meropenem and imipenem, carbapenemase production was not seen in all isolates in both PCR and CIM tests. These results indicate that carbapenem resistance was mediated by different carbapenemase genes or mechanisms. The evaluation of carbapenem resistance is critical for the prevention of healthcare-associated infection.

Key Words: *Acinetobacter baumannii*, carbapenemase, CIM, PCR.

ÖZET

Karbapenem dirençli *Acinetobacter baumannii* izolatlarında karbapenemaz üretiminin fenotipik ve genotipik yöntemlerle araştırılması

DABAN KANABI RASHID

Yüksek Lisans, Tokat Gaziosmanpaşa Üniversitesi Sağlık ve Fen Bilimleri Enstitüsü
Tıbbi Mikrobiyoloji Anabilim Dalı

Tez Danışmanı: DOÇ. Dr. Umut Safiye ŞAY COŞKUN

Karbapenem dirençli *Acinetobacter baumannii*'nin neden olduğu hastane enfeksiyonları tüm dünya için ciddi bir tehdit oluşturmaktadır. Özellikle mortalite oranı yüksek olan pnömoni ve kan dolaşımı enfeksiyonları, bağışıklık sistemi baskılanmış bireylerde en sık görülen enfeksiyonlardır. Tıbbi tedavi tekniklerinde sürekli ilerlemelere rağmen yeni antibiyotiklerin geliştirilmesinde çok az gelişme olmuştur. Artan antibiyotik direnci nedeniyle karbapenem dirençli *A. baumannii* enfeksiyonlarını tedavi etmek için kullanılabilecek antibiyotik seçenekleri kısıtlıdır.

Bu çalışmaya Tokat Gazi Osmanpaşa üniversite hastanesi farklı kliniklerden alınan (püy, yara sürüntüsü, kateter, balgam ve idrar) örneklerinden toplam 80 isolat dahil edildi. İzolatların identifikasyonu ve antibiyotik duyarlılık testi Vitek-2 (bioMerieux, France) otomatize sistemi (bioMerieux, Fransa) ile yapıldı. Karbapenemlere direnci belirlemek için ayrıca disk difüzyon yöntemi kullanıldı. Karbapenemaz enzimini göstermek için karbapenemaz inaktivasyon yöntemi ve karbapenem dirençli genleri tanımlamak için polimeraz zincir reaksiyonu kullanıldı. İzolatların en sık beyin cerrahisi kliniklerinden gönderilen örneklerden tanımlandığı görüldü. Ayrıca izolatlar en sık balgam örneklerinden üretildi. Karbapenem inaktivasyon testi ile karbapenemaz üretimi %63 (n=50) pozitif olarak tespit edildi. Karbapenemase genleri araştırıldığında izolatların 14/38'i (%33) IMP, 11'i (%14) blaOXA-48, 9(11%) NDM ve 8(%10) izolatta da NDM+KPC genleri pozitif bulundu. NDM ile birlikte olan izolatlar dışında hiçbir izolatta KPC saptanmadı.

İzolatlarda karbapenemaz üretiminin fenotipik tespitinin aksine, izolatların 50/80'i (%63) PCR ile pozitifti. Bu çalışmada tüm izolatlar meropenem ve imipeneme dirençli olmasına rağmen hem PCR hem de CIM testlerinde tüm izolatlarda karbapenemaz üretimi görülmedi. Bu sonuçlar, karbapenem direncine farklı karbapenemaz genleri veya mekanizmalarının aracılık ettiğini göstermektedir. Karbapenem direncinin değerlendirilmesi, sağlık bakımıyla ilişkili enfeksiyonun önlenmesi için kritik öneme sahiptir.

Anahtar sözcükler: *Acinetobacter baumannii*, karbapenemaz, CIM, PCR.

CONTENTS

LIST of TABLES

LIST of FIGURES

SYMBOLS AND ABBREVIATIONS

Adcs: *Acinetobacter*-derived cephalosporinases

Ampc: Ampicillin-hydrolyzing cephalosporinase

Bap: Biofilm-associated proteins

Blsa: Blue-light-sensing protein A

CRAB: Carbapenem-resistant *Acinetobacter baumannii*

CSF: Cerebral spinal fluid

CTX-M: Cefotaximase-Munich

DNA: Deoxyneuclotidic acid

ESBLS: Extended-spectrum beta-lactamasemases

ESKAPE: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa and Enterobacter*

G+C: Guanine+Cytocine

ICU: intensive care unit

IMP: Impenemase

Isaba1: İnsertion sequence ısaba1

KPC: *Klebsiella pneumonia* carbapenemase

LPS: Lipo polysaccharide

MBL: Metallo beta lactamase

MDR: Multidrug resistant

MIC: Minimum inhibitory concentrations

Mn: Manganase

NDM: New delhi metallo—lactamase

OMP: Outer membrane proteins

OPD: Obstructive pulmonary disease

OXA: Oxacillinase

PBPs: Penicillin binding proteins

SE: Asian South east Asia

SHV: Sulf-hydryl reagent variable

TSS: Type secretion system

UTI: Urinery tract infection

VAP: Ventilator associated pneumonia

VEB-1: Vietnamese extended-spectrum β-lactamase

VIM: Verona Integron-encoded MBL

WHO: World Health Organization

XDR: Xtend drug resistant

Zn: Zinc

1. INTRODUCTION

Acinetobacter baumannii has emerged in recent decades as a nosocomial pathogen with a high worldwide pathogenicity. Its clinical significance has been largely fueled by its amazing capacity to acquire or upregulate a variety of resistance determinants, making it one of the most effective MDR organisms, defying current antibiotic treatment (Clark et al., 2016). Additionally, to this remarkable resistance acquisition, *A.baumannii* possesses a variety of mechanisms for surviving in a variety of conditions, enhancing its capacity for hospital spread (Peleg et al., 2008). Attributable mortality rates for patients with *A. baumannii* healthcare-associated infections, the most prevalent of which are ventilator-associated pneumonia and bloodstream infections, vary from 5% in regular hospital wards to 54% in critical care units (ICU). Rising reports of *A. baumannii* infections acquired in the community (Halat and Moubareck., 2020).

Nosocomial and community-acquired infections are both on the rise as a result of the rise in multidrug-resistant (MDR) bacteria. Indeed, the World Health Organization (WHO) has designated antibiotic resistance as one of the three most critical issues affecting human health today (Howard et al., 2012). While most antibiotics were considered to be responsive to *A. baumannii* in the 1970s, the bacterium now seems to have considerable resistance to most first-line antibiotics (héritier et al., 2066). *A. baumannii* is one of the most difficult gram-negative bacteria to control due to its multidrug resistance, which is one of the major medical issues of the 21st century (Manchanda et al., 2010). *A. baumannii* is one of these organisms and a member of the ESKAPE group of six antibiotic-resistant bacteria are the most common causes of antibiotic-resistant illnesses (Husni et al., 1999).

Carbapenem-resistant the world health organization considers *A. baumannii* to be the most urgently needed novel treatment priority pathogen (Shlaes and Bradford,. 2018). Carbapenems were effective against the majority of isolate up until recently, however, isolated reports of resistance date back to the early 1990s (Richet et al., 2001). Resistance to carbapenems has emerged in one of the most significant Gram-negative bacilli in the previous decade. Carbapenemases, efflux pumps with enhanced activity, and porin loss are the three most frequent pathways for carbapenem Resistant development (Martinez., 2008).

Nowadays, antibiotic resistance is the most serious problem in the world since it affects patients, healthy individuals, and people of all ages. *A. baumannii* is a major concern that has been the most commonly acquired infection in hospitals over the last two decades. This study will isolate carbapenem-resistant *A. baumannii* strains in Turkey in order to control nosocomial infections caused by this bacteria when it is determined to be resistant to carbapenems and gain new knowledge about those strains that will be detected in Turkey's hospitals in order to reduce mortality and morbidity caused by *A. baumannii* because carbapenem antibiotics play a critical role in the treatment of hospital-acquired infections caused by *A. baumannii*.

1.1 Genus and species of *Acinetobacter spp.*

As *Micrococcus calco-aceticus* was identified for the first time in 1911 by the Dutch scientist Beijerinck. It was found in soil using enhanced minimum medium with calcium acetate. Brison and Prevot introduced the present name *A.*, which was given its current name in 1954 (from the Greek "akinetos", meaning "non-motile") to differentiate non-motile bacteria from motile bacteria within the genus *Achromobacter*. By 1968, the genus *Acinetobacter* had gained widespread acceptance after the work of Baumann et al. (Peleg et al., 2008). Following Bouvet and Grimnot's DNA-DNA hybridization studies in 1986 (Raffaele et al., 2011).

Currently, the *Acinetobacter* genus has 33 distinct genospecies. genospecies 2 (*A. baumannii*), *A. baumannii* is the most prevalent and most resistant species of *Acinetobacter*, making up more than 90% of all *Acinetobacter* species isolates. Other Acinetobacter species (*Acinetobacter* genospecies 3, *Acinetobacter* genospecies 13 and *Acinetobacter lwoffii*, *Acinetobacter ursingii*,), They've been linked to human illness and are more sensitive to antimicrobials (Garnacho and Amaya., 2010).

1.2 Microbiological properties

Acinetobacter spp. are characterized gram-negative, oxidase-negative, catalase-positive, nonfermenting coccobacilli. They have a G+C composition of 39% to 47% in their DNA. However, since De-staining the organisms is often a difficulty, they are frequently misidentified as Gram-positive. Solid media often employed in clinical microbiology labs, such as sheep blood agar or tryptic soy agar, are excellent growth medium for human-derived *Acinetobacter* species when incubated at 37°C. This organism produces smooth, occasionally mucoid, grayish white colonies; Frequently, the *Acinetobacter* species produce small and semi-transparent colonies (Peleg et al., 2008).

1.3 Epidemiology

A. baumannii is found in a variety of health care settings and is a very successful colonizer of humans in hospitals. Due to its environmental tolerance and a diverse repertoire of resistance determinants, it is a successful nosocomial pathogen (Nordmann., 2004). As a result, *A. baumannii* has emerged as a leading cause of worldwide epidemics. exhibiting ever-increasing resistance rates. MDR *A. baumannii* infections have been reported in hospitals across Europe, North America, Argentina, Brazil, and SE Asian. These MDR strains typically spread rapidly, causing epidemics that cover whole cities, nations, and continents (Perez et al., 2007).

There has been evidence of the importation of MDR strains from places with high antimicrobial resistance rates, such as Spain, to areas with low rates, such as Norway (Onarheim et al., 2000). Recently, reports of military and civilian individuals from the UK and the United States returning from Iraq and Afghanistan operation and harboring illnesses caused by MDR *A. baumannii* have garnered considerable attention (Perez et al., 2007).

An increased risk of *A. baumannii* acquisition is associated with old age, the presence of significant underlying conditions (such as immune suppression), invasive operations, major trauma or burn injuries, the presence of in-place catheters, mechanical breathing, and a protracted hospital stay. Multidrug-resistant (MDR) *A. baumannii* can be acquired through the use of broad-spectrum antibiotics, such as third-generation cephalosporins, fluoroquinolones, and carbapenems, as well as prolonged mechanical ventilation, prolonged ICU or hospital stays, and contact with infected or colonized patients (Karageorgopoulos and Falagas., 2008).

Carbapenem resistance rates in *A. baumannii* have recently risen sharply. According to the National Healthcare Safety Network data in 2006–2007, *A. baumannii* was found to be resistant to carbapenems in 33% of cases in the United States. Carbapenem-resistant *A. baumannii* (CRAB) was also shown to be growing in hospital-acquired infections, with an overall rate of 3% of hospital-acquired infections, according to these findings (8.4 percent of VAP, 2.2% of central line associated bloodstream infections, 1.2% of catheter-associated urinary tract infections and 0.6% of surgical site infections) (Hidron et al., 2008). CRAB has become a major danger to the antibiotic era because of its rapid growth and evolution. After that, we'll discuss CRAB's resistance mechanisms, infection control, as well as monitoring and treatment of CRAB infections (Pogue et al., 2013).

1.4 Pathogenesis and immunity

1.4.1 Adhesion and attachment

The adhesion of bacteria to epithelial cells is a first stage in infection and colonization. Human skin and mucous membranes may colonize and sustain *Acinetobacter* species for days or weeks, according to epidemiological research (Cho at el., 2009). In vitro studies have shown that *A. baumannii* adheres to human bronchial epithelial cells. dhesion molecules, such as OmpA, Omp33–36, Bap and cell fibronectin, play a major role in this phenomenon (Akrami and Namvar., 2019).

1.4.2 Motility

Certain bacteria have motility as a role in their capacity to infect. In the past, *A. baumannii* was considered non-motile due to its absence of flagella. However, research has shown that this organism with its twitching motility, it is able to survive during infection and disseminate on hospital surfaces. Extending and retracting actions of type IV pili are used to move cells around in the media. Creating biofilms and transferring genes are two other functions of these pili (Halat and Moubareck., 2020). The T gene in pili is required for bacterial twitching motility. Mutant strains' motility is reduced by 54% as a result of this gene's inactivation. As a result, the motility of *A. baumannii* may be reduced by increasing the content of agar. Cell population and free iron concentration play crucial roles in the motility of *A. baumannii* (Clemmer et al., 2011).

1.4.3 Outer Membrane Proteins (OMPs)

Pathogenicity in host cells, compatibility, and antibiotic resistance are linked to Gram-negative bacteria's outer membrane proteins. This factor is required for binding, bacterial invasion, and the prevention of lysis caused by complement system activation.

Through mitochondrial targeting, it has been related with epithelial cell apoptosis (Lee et al., 2010).

A. baumannii's outer membrane protein A (OmpA, originally Omp38) is a well-known virulence factor, Which is the most common kind of OMP (Morris et al., 2019). Antimicrobial resistance in *A. baumannii* is also mediated by Omp *A. baumannii*. Additionally, removing the ompA gene reduces the minimum inhibitory concentrations (MICs) of numerous antibiotics considerably (nalidixic acid, chloramphenicol, and aztreonam), that OmpA participates in the expulsion of antibiotics through the outer membrane and connects to efflux mechanisms in the inner membrane Figure. (1) (Lee et al., 2017).

Another outer membrane protein linked with *A. baumannii* cytotoxicity is the 33- to 36-kDa Omp protein (Omp33-36), which functions as a water passage channel (Rumbo et al., 2014). Antibiotic resistance is also linked to Omp33-36. Resistance to carbapenem antibiotics (meropenem and imipenem) in *A. baumannii* strain JC10/01, This strain's MICs for imipenem and meropenem are significantly reduced due to the deletion of episomal expression of Omp33-36 and Omp33-36 (Perez et al., 2005).

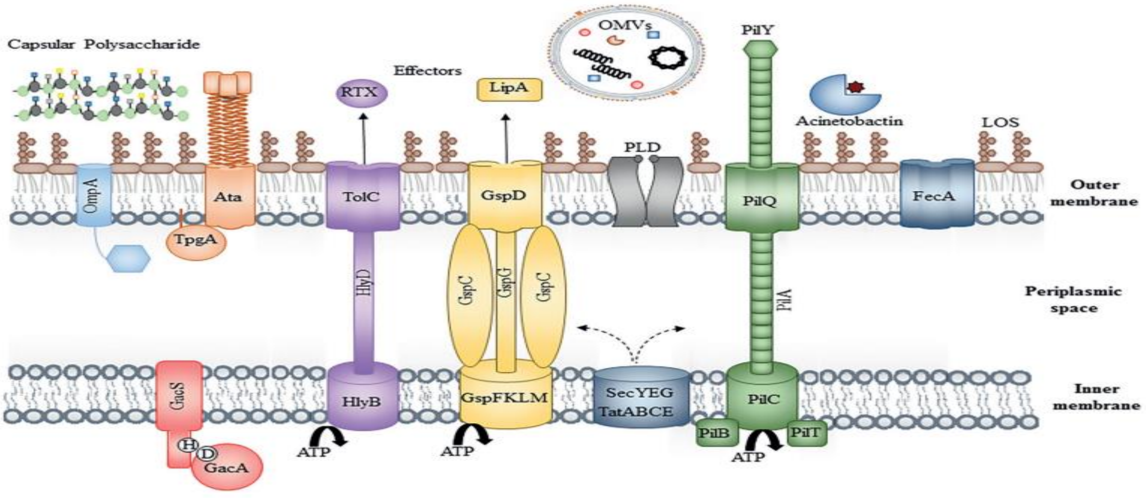

Figure 1: bacterial vitulence factors. Including outer membrane protein A, capsule polysaccharide, the type II, IV and V secretion system, phospholipase D, iron aquistition, lipid oligosaccharide, and outer membrane vesicles

1.4.4 Outer membrane vesicle

OMVs are nanovesicles containing outer membrane proteins, nucleic acids, LPS, and lipids in the form of a round shape. Quorum sensing, transmission of virulence factors, phagolysosome fusion inhibition, gene transfer, and the development of biofilms are all examples of their roles figure. (1) (Akrami and Namvar., 2019).

1.4.5 Cell envelope factors (Lipopolysaccharide and Capsule)

Cell wall polysaccharide (LPS) is a key component in the illness process in Gram-negative human infections (Knapp et al., 2006). Resistance to polymyxin antimicrobial peptides, complement activation, responses, and synergistic interactions with exopolysaccharide capsule are all dependent on bacterial LPS (Akrami and Namvar., 2019). LPS is critical for *A. baumannii* pathogenicity and survival figure. (1) (Luke et al., 2010).

In order to resist cationic antimicrobial peptides and defend bacteria from the host's innate immune response, the capsule produces a protective coating on the extracellular surface. This improves the in vivo survival of bacteria (Morris et al., 2019). A conserved gene cluster known as the K locus, found in numerous isolates from individuals with *A. baumannii* infections, may be responsible for determining the formation of capsular polysaccharides in these isolates. Antimicrobial resistance in *A. baumannii* seems to be linked to capsular polysaccharides, according to one research. Capsular polysaccharide-deficient mutants had a decreased inherent resistance to peptide antibiotics. Additionally, antibiotics stimulate the formation of capsular polysaccharides. Increased capsule synthesis after antibiotic exposure is dependent on transcriptional increases in K locus gene expression, which are controlled by the bfmRS two-component regulatory system, according to this research. Virulence factor BfmS also contributes to biofilm development, eukaryotic cell adhesion, and resistance to human serum. (Lee et al., 2017).

1.4.6 Protein secretion system

Protein secretion from cell surface structures in *A. baumannii* enables the pathogen to interact with the environment and hosts, making it an appealing treatment target figure. (1) (Bentancor et al., 2012).

1.4.6.1 Type II secretion system

In Gram-negative bacteria, the protein complex T2SS When compared to type IV pili systems, which have the same structure, an appendage that is common (Korotkov et al., 2012). T2SS is a two-step secretion process that relies on either the twin arginine transport (Tat) system or the general secretory (Sec) system to move substrate to the periplasm prior to secretion (Eijkelkamp et al., 2014). In order to export several effector proteins, *A. baumannii* utilizes a type II secretion system (T2SS) (Sandkvist., 2001). Lipase, elastase, alkaline phosphatase, and phospholipase enzymes. These are crucial for *A. baumannii* pathogenicity, are among the effector proteins (Elhosseiny and Attia,. 2018).

1.4.6.2 Type IV secretion system

Plasmids, DNA, and other mobile genetic components are transferred conjugatively through the Type IV secretion system (T4SS). *Acinetobacter* T4SS has been the subject of just three studies. Although these traits are important for the transmission of genetic material, such as antibiotic resistance determinants, their involvement in host-pathogen interactions has not yet been determined (Morris et al., 2019).

1.4.6.3 Type V secretion system

Type V secretion system (T5SS) (autotransporters) is the simplest and most prevalent secretion system in Gram negative bacteria (Henderson et al., 2000). The trimeric autotransporter (Ata) was the first secretion system found in *A. baumannii* (Weber et al., 2017). In *A. baumannii* ATCC 17978, the Ata autotransporter secretion system plays a critical role in pathogenicity, biofilm development, binding to extracellular matrix proteins, and adhesion. The researchers discovered that Ata is associated with 58 percent of clinical isolates, but not with global strains (Eijkelkamp et al., 2014).

1.4.6.4 Type VI secretion system

The T6SS is used by many bacteria to release effector proteins that aid in the colonization of eukaryotic hosts or to annihilate rival bacteria (Shneider et al., 2013). When *Acinetobacter* uses its T6SS to attack other bacteria, it secretes a wide variety of toxins, including peptidoglycan hydrolases and nucleases as well as those that attack cell membranes (Fitzsimons et al., 2018).

1.4.7 Biofilm formation

Acinetobacter is able to colonize on both biological and abiotic surfaces, forming biofilms. Colonization, microcolonies and exopolysaccharides secreted by bacteria ultimately lead to biofilm formation. It is also possible for biofilm to influence drug resistance and the host's immune system to escape. Two-component regulatory systems and quorum sensing are used to regulate this process. Among the most significant components in biofilm development are outer membrane proteins, pili, Bap (Biofilm binding protein) and extracellular polysaccharide (Akrami and Namvar., 2019). *A. baumannii* is 'marked by the development of biofilm-associated proteins (BapAb) that are homologous to the Bap protein found in Staphylococcus aureus and have the ability to assemble in response to stressful environmental circumstances (Loehfelm et al., 2008).

1.4.8 Enzymes

Acinetobacter's hydrolytic enzymes include phospholipases C and D. Lipolytic enzymes catalyze the release of phospholipids. The lysis of host cells by these enzymes' aids in the pathogenesis of Gram-negative bacteria. Bacterial invasion will be facilitated by the breakdown of phospholipids in mucosal tissues (Akrami and Namvar., 2019). Enzyme information for *A. baumannii* continue to advance; Virulence factor CpaA, an adamalysin-like protease specific to glycans, has recently been revealed to suppress blood coagulation by inactivating factor XII. As a result, CpaA inhibits the production of thrombi at intravascular regions, hence increasing A's dispersion capability *A. baumannii* (Waack et al., 208).

1.4.9 Iron Uptake System:

Iron acquisition mechanisms are used by pathogenic bacteria to acquire the host's low amount of free iron. Iron absorption is facilitated by binding to certain receptors, transferring proteins, and siderophores (Eijkelkamp., 2011). Pathogenic bacteria generate siderophores, high-affinity iron chelators that can be separated into catecholates, carboxylates, and hydroxamates depending on the iron chelating ligand. A catechol-hydroxamate siderophore, is one of *A. baumannii*'s iron acquisition methods (Hasan et al., 2015).

1.4.10 Zinc and Manganese uptake system

Metals like as manganese (Mn2+ and Mn) and zinc (Zn2+ and Zn) are chelated by calprotectin, an innate immunological metal-chelating protein, to suppress bacterial development (Corbin et al., 2008). Similar to iron, zinc plays a crucial role in biology by functioning as a natural catalyst and cofactor for a variety of proteins (Hood and Skaar, 2012). In the presence of this immunological protein, *A. baumannii* may induce disease in vivo. An important finding is that limiting zinc reduces the imipenem MIC of MDR *A. baumannii* to levels below which the organism is clinically resistant to imipenem. probably due to the fact that the hydrolysis activity of many carbapenemases is a metalloenzyme that requires Zn. Zn metallochaperone ZigA was discovered in *A. baumannii* in addition to the ZnuABC system. *Baumannii* (Lee et al., 2017).

1.4.11 Blue-light sensing protein A

The *A. baumannii* pathogenicity may be affected by the blue-light-sensing protein A (BlsA). However, if the bacteria are exposed to blue light, they are unable to form biofilms (Richards et al., 2015).

1.4.12 Quorum sensing

A bacterium's capacity to detect, react and communicate with its neighbors is vital to its survival. bacteria recognize and react to hormone-like molecules like acyl homoserine lactone to regulate a wide range of traits, including motility and biofilm formation. N-(3-hydroxydodecanoyl)L-HSL, which is synthesized in *A. baumannii* by abaI in conjunction with the abaR LuxR homolog, regulates biofilm development (Morris et al., 2019).

1.5 Clinically significant

A. baumannii may cause a wide variety of diseases, including pneumonia, bacteremia, urinary tract infections, meningitis, and skin and soft tissue, the latter of which is the most often reported illness in both settings (Dexter et al., 2015). Long-term hospital stays, the presence of mechanical ventilation or indwelling catheters, advanced age, immune suppression, burns or major trauma, comorbid diseases, previous antibiotic use, and invasive procedures, are all risk factors for developing an *A. baumannii* infection in critically ill patients. It's hard to figure out how many people who are very sick and have *A. baumannii* infections die because their prognosis is already bad. In contrast, crude morality rates have ranged between 23% and 68% (Morris et al.,2019). In regions where the temperature is hot and humid, community-acquired illnesses have an unique and severe clinical condition. These infections are more prevalent in those with pre-existing health disorders, such as diabetes mellitus and chronic obstructive pulmonary disease (OPD), as well as in those who are heavy smokers or consume excessive amounts of alcohol (Falagas et al 2007).

A. baumannii, which persists on contaminated hospital equipment or in the hands of healthcare workers who have come into touch with the organism as a result of interaction with a colonized patient, is considered to be the most common source of infection (Maragakis et al., 2004).

1.5.1 Respiratory tract infection

Hospital-acquired pneumonia is one of the most common infections caused by *A. baumannii,* and these infections frequently occur in patients receiving mechanical ventilation in the intensive care unit because of the colonization of *A. baumannii* in the airway via environmental exposure, which plays a role in the progression of pneumonia (Dijkshoorn et al., 2007). *A. baumannii*-induced ventilator-associated pneumonia has been reported to have a mortality rate of between 40% and 70%, however the mortality directly related to *A. baumannii* infection has been the topic of contention (Fagon et al., 1996).

1.5.2 Bacteremia

A. baumannii is responsible for 1.5 to 2.4 percent of bloodstream infections in the hospital. *A. baumannii* may cause bacteremia by infecting a vascular catheter or the respiratory tract. intensive care, mechanical breathing, previous surgery, past use of broad-spectrum antibiotics, immunosuppression, traumatic injury or malignancy, central venous catheter, invasive procedures and extended hospitalization are all risk factors for *Acinetobacter* bloodstream infection. *Acinetobacter baumanii* is the causative agent in one-third of septic shock cases in patients with nosocomial blood stream infections, with fatality rates ranging from 20% to 60%. This mortality rate is higher when *Acinetobacter bacteremia* is associated with nosocomial pneumonia (Kanafani and Kanj., 2013).

1.5.3 Urinary Tract Infection

Despite the fact that most studies on *A. baumannii* infections have concentrated on pneumonia and bloodstream infections, current reports indicate that one out of every five *A. baumannii* strains has been identified from urine locations. Occasionally, *A. baumannii* linked to urinary tract infections (UTIs), particularly urinary catheters, In one research, 1.6 percent of intensive care unit-acquired UTIs were attributed (Moubareck and Hammoudi., 2020).

1.5.4 Meningitis

Nosocomial meningitis caused by *A. baumannii* continues to be a growing hazard in intensive care neurosurgical units, with mortality approximately 70%, particularly in patients with indwelling ventriculostomy tubes or CSF fistulae who are taking post-surgical antibiotics (Siegman et al., 1993). In the biggest case series of post-neurosurgical *A. baumannii* meningitis published in 2019, it was discovered that 21% of isolates showed an XDR phenotype, meaning they were solely susceptible to colistin and tigecycline. Additionally, patients over the age of 40, those with an external ventricular drain, and those with elevated cerebrospinal fluid white blood cell counts were all at risk for mortality from *A. baumannii* in the neurosurgical ICU (Sharma et al., 2019).

1.5.5 Endocarditis

Endocarditis caused by *Acinetobacter* species is an uncommon occurrence in both native and prosthetic heart valves, and mortality tends to be more apparent in the case of native valve endocarditis than in the case of prosthetic valve endocarditis (Gradon et al., 1992).

1.5.6 Burn Infection

An important cause of burn infections is *A. baumannii.* Due to the high prevalence of multidrug resistance and the limited penetration of certain medicines into burn sites, these infections may be particularly difficult to treat. Infection and colonization of burns may be difficult to distinguish (albrecht et al., 2006).

1.5.7 Other Infection

Surgical and traumatic wounds may be contaminated with *Acinetobacter*, which can cause severe soft tissue infection and even osteomyelitis (Davis et al., 2005). *Acinetobacter* infections in surgical wounds are commonly associated with the presence of prosthetic material and frequently need considerable debridement. *Acinetobacter* has been linked with a very small number of community- or hospital-acquired skin infections, including folliculitis and cellulitis, as well as necrotizing fasciitis and skin abscesses (Glew et al., 1977).

1.6 Antibiotic resistant

1.6.1 The Carbapenems and *A. baumannii*

The increase in the number of illnesses caused by *A. baumannii* over the last several decades is a reason for worry, given the challenges associated with administering effective antibiotic therapy. Due to the species' inherent characteristics, such as chromosomally encoded lactamases, a highly permeable barrier, and the potential of mobile genetic elements to acquire and retain resistance determinants, the number of effective antibiotics against some isolates has been drastically reduced, in some cases to nothing (Valencia et al., 2009). Over the last few decades, a lot of *A. baumannii* strains have become resistant to a lot of different types of antibiotics. This has led to the abolition of aminoglycosides, penicillins, tetracyclines, cephalosporins and quinolones as effective treatments for a lot of *A. baumannii* strains. There is now just one main type of antibiotics that can be used to treat infections with *A. baumannii,* carbapenems. Carbapenems, which include imipenem, meropenem, and doripenem, have traditionally been regarded as the agents of choice for treating *A. baumannii* infections according to their efficacy and safety (Lee et al., 2017). However, resistance to carbapenems has increased in recent years, which concerns this treatment option as well. Carbapenems are a subclass of beta-lactamasem antibiotics that exhibit the largest variety of action within this class. Imipenem, Streptomyces cattleya developed a stable thienamycin derivative in the 1970s, was the first carbapenem to be licensed for usage in 1985. Meropenem was developed in 1993, ertapenem was released in 2001, and doripenem was introduced in 2007 (Livermore et al., 2009). As with other beta-lactam antibiotics, carbapenems attach to the penicillin-binding proteins found in bacteria, inhibiting the final transpeptidation of the peptidoglycan in the cell wall, eventually resulting in the cell destroying its own cell wall and dying. In the presence of most beta-lactamasemases, carbapenems are resistant to hydrolysis of the link between positions 4 and 7 because they include a trans-a-1-hydroxyethyl group at position 6, Figure. (2). Since then, there has been an increase in the number of carbapenem-resistant *A. baumannii* infections. Due to the propensity of *A. baumannii* isolates to resist carbapenem activity through a variety of mechanisms detailed in the next sections, there is no apparent therapeutic option for infections caused by this bacterium (Zhanel et al., 2007).

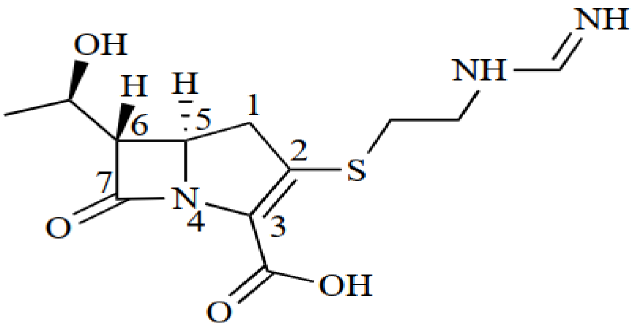

Figure 2: Imipenem's chemical structure. The penem and beta-lactamasem ring locations are numbered.

1.6.2 Beta-lactamase

A significant mechanism of antibiotic resistance in *A. baumannii* is the inactivation of beta-lactams by beta-lactamases. On the basis of their sequence homology, beta-lactamases are classified into molecular classes A, B, C, and D (Jeon et al., 2015). A beta-lactamase belonging to all four classes has been found in *A. baumannii.* Recent studies have shown that *A. baumannii* is naturally able to take in foreign DNA and that its genome is full of foreign DNA, which shows that horizontal gene transfer happens a lot in this bacterium (Traglia et al., 2014). *A. baumannii's* inherent competence is further enhanced by albumin, a major blood protein. It may thus be possible to identify several different types of beta-lactamasemases in this serious human disease due to *A. baumannii's* inherent competence (Lee et al., 2017).

1.6.2.1 Ambler class A beta-lactamase

Ambler class A beta-lactamases hydrolyze penicillins and cephalosporins in a variety of ways and are often sensitive to beta-lactamasemase inhibitors. The majority of extended-spectrum beta-lactamasemases (ESBLs) are produced by this enzyme family, as it contains the CTX-M, SHV, and TEM enzyme families. *A. baumannii* seems to have a rather high prevalence of class A beta-lactamasemases. The gene encoding the enzyme PER-1 has been identified in a number of isolates from the United States of America, Turkey, and Korea (Hujer et al., 2006, walsh et al., 2005). VEB-1 enzymes are present in a considerable number of isolates from Belgium and France. There have also been reports of isolates encoding TEM, SHV, and CTX-M-type enzymes. *A. baumannii* is resistant to cephalosporins and penicillins due to the inherent class C lactamases, hence these medications are not normally regarded as acceptable for treating *Acinetobcter baumannii* infections. *A. baumannii*'s high incidence of class A beta-lactamasemases is thus not a major health issue in itself; nevertheless, the possibility that this species may serve as a hospital reservoir for mobile resistance genes may be more concerning (Evans et al., 2013). The Ambler class A carbapenemases (SME, NMC, and IMI, and GES) for *A. baumannii* have not yet been identified (Queenan and Bush., 2007).

1.6.2.2 Ambler class B beta-lactamase

Class B Ambler beta-lactamases (alternatively referred to as metallo-beta-lactamasemases) are different from class A, C, and D enzymes in that their active site contains one or two metal ions, often zinc, rather than a serine residue. A class B beta-lactamase, MBLs can hydrolyze carbapenems and all other beta-lactam antibiotics except aztreonam, and beta-lactamases inhibitors do not affect (Walsh et al., 2005). Despite the fact that MBLs are less abundant in *A. baumannii* than OXA-type carbapenemases, their hydrolytic activity against carbapenems is substantially stronger (100- to 1,000-fold). Almost all of the beta-lactamasems can be hydrolyzed except for the monobactam aztreonam, which could help scientists identify these enzymes in the lab. The five MBL groupings that have been characterized to date, Only IMP, VIM and

SIM types have been discovered in *A. baumannii*. The occurrence of both OXA- and MBL-type enzymes in the same strains has been found in many areas, including Spain, Singapore, Greece, and Australia (Peleg et al., 2008). Different from other types of OXA-type enzymes, the majority of MBLs may be found in the genetic structures known as integrons, which have been designed to make it easier for resistance-determinants to be acquired and expressed (by means of one common promoter). Most of the acquired MBL genes in *A. baumannii* came from class 1 integrons, which often have a lot of resistance gene cassettes, especially those that make aminoglycoside-modifying enzymes (Houang et al., 2003, Lee et al., 2005). integron-carrying *A. baumannii* strains have been discovered to be substantially more resistant to antibiotics than strains lacking integrons (Gu et al., 2007).

1.6.2.3 Ambler class C beta-lactamase

Almost all strains of *A. baumannii* have chromosomally encoded AmpC cephalosporinases, which are also called Acinetobacter-derived cephalosporinases (ADCs). beta-lactamase inhibitor, Cephalosporin, penicillin, and Cephamycin (cefotetan and cefoxitin) combinations resistance are mediated by these enzymes, however clavulanate and sulbactam are not affected by them (Moubareck and Halat et al., 2020). Activation of the AmpC gene is induced in other Gram-negative organisms. However, in *A. baumannii*, blaADC genes are activated by inserting the insertion sequence ISAba1, which acts as a promoter for gene expression. The extended-spectrum cephalosporins may be resistant to the enzymes when they are overexpressed (Corvec et al., 2003).

1.6.2.4 Ambler class D beta-lactamase

Because Class D beta-lactamases hydrolyze isoxazolylpenicillin and oxacillin faster than benzylpenicillin, they are known as OXAs (oxacillinases) (Lee et al., 2015). There are more than 400 currently recognized varieties of OXA-type enzymes, and many of these enzymes include carbapenemase activity as part of their repertoire of activities. A significant mechanism of carbapenem resistance in *A. baumannii* is the existence of carbapenem-hydrolyzing class D beta-lactamases, or MBLs (Lan and lin., 2014). The carbapenem-hydrolyzing OXA-type enzyme was initially discovered in a clinical *A. baumannii* strain in Edinburgh, Scotland, in 1985. Plasmid-encoded resistance determinant (formerly known as "ARI-1") proved to be transferrable, and the gene was eventually sequenced and designated "blaOXA-23." (Peleg et al., 2008). Carbapenem-hydrolyzing OXAs are divided into five family members: OXA-23-like, OXA-40-like, OXA-51-like, OXA-58-like, and OXA-143 enzymes encoded by *A. baumannii* isolates. The genes encoding the OXA-51-like enzymes are believed to be inherent to the species but the genes representing four of these families are believed to have come from a different source, according to current research (Poirel et al., 2010). An example of this is that when ISAba1 is added to the promoter sequence of the promoter for blaOXA-23, *A. baumannii* overproducing blaOXA-23, blaOXA-51, or blaOXA-58 (Turton et al., 2006).

1.6.2.5 OXA-23 cluster (includes OXA-23, OXA-27 & OXA-49)

The blaOXA-23 gene cluster in *A. baumannii* contains two closely similar enzymes, OXA-27 and OXA-49 (Peleg et al., 2008). OXA-27 and OXA-49 are subgroups that vary from OXA-23 by two amino acid changes (Scaife et al., 1995). In addition to hydrolyzing carbapenems, OXA-23, OXA-27, and OXA-49 enzyme kinetic studies have shown that these enzymes are also active against oxyiminocephalosporins, aminopenicillins, and oxacillin (Paton et al., 1993, afzal-shah et al., 2001). The three enzymes have different activities, with OXA-27 being more active against carbapenems but less active against cephaloridine and oxacillin than OXA-23. OXA-23 leads to resistance to ticarcillin, meropenem, imipenem, and amoxicillin in isolates expressing the enzyme. It was found that when the carbapenem-resistant strain blaOXA-23 was transformed into a susceptible *A. baumannii* isolate, it resulted in relatively low levels of resistance, while moderate levels of resistance were obtained when the resistant strain was transformed into a strain overexpressing the RND-type AdeABC efflux pump. High levels of carbapenem resistance were only achieved when susceptible isolates were transformed with the whole native plasmid harboring blaOXA-23, with the greatest finding being related to AdeABC over-expression (Evans et al., 2013). This shows that in order for isolates to reach clinically important levels of carbapenem resistance, other resistance mechanisms in addition to OXA-23 expression are necessary. It has been discovered that members of the blaOXA-23 gene family are present in a variety of distinct *Acinetobacter* species, Proteus mirabilis and Klebsiella pneumoniae (Bogaerts et al., 2006). The genes have been reported on both chromosomes and plasmids, and their G+C content of 37.6-37.9 percent falls outside the A. baumannii genome's range of 39-47 percent (Bergogne et al., 1996).

1.6.2.6 OXA-24 cluster (includes OXA-24, OXA-25, OXA-26 & OXA-40)

A few years after OXA-23 was found, the first member of a second group of OXA-type beta-lactamasemases was found in *A. baumannii* isolates from a 1997 incident in a hospital in Spain (Bou et al., 2000). The enzyme was initially referred to as OXA-24, but has now been renamed OXA-40. Five following enzymes exhibited more than 99 percent amino acid similarity to OXA-40. The OXA-25 enzyme was discovered in a Belgian strain, whereas OXA-26 was discovered in a Spanish isolate that was isolated between 1995 and 1997 (Afzal et al., 2001).

The kinetics of three OXA-40-like enzymes have been investigated: OXA-40, OXA-25, and OXA-26. The enzymes were affinity to hydrolyze carbapenems and penicillins, as well as a number of cephalosporins, although their activity was limited. OXA-40 had the greatest degree of activity against carbapenems, and all three enzymes hydrolyzed meropenem more efficiently than imipenem. OXA-40's crystal structure was reported in 2007 (Evans et al., 2013), This was the first time the structure of an *A. baumannii* OXA-type beta-lactamasemase has been shown. This work identified many structural features of the enzyme that may help it choose its target substrates. Because the fundamental structural properties of the region around the active site of OXA-40 are typically preserved, comparing it to those of other class D beta-lactamasemases could not give an explanation for OXA-40's capacity to hydrolyze carbapenems while showing a diminished ability to hydrolyze oxacillin (Santillana et al., 2007. Sheri et al., 2010).

1.6.2.7 OXA-51 Cluster

Intrinsic enzymes, known as OXA-51-like enzymes, are identified in *A. baumannii* isolates in addition to OXA-type beta-lactamases that are acquired externally. An enzyme called OXA-51 was found in Argentinean carbapenem-resistant isolates from 1996 (Evans et al., 2013). One of the biggest beta-lactamasemases has now been identified after 72 more sequence variations were discovered. In recent studies, the blaOXA-51-like genes have often been reported to be non-transferable across isolates and to be localized on the chromosome in limited studies till recently. However, there are currently several notable exceptions. Firstly, two papers reported in 2006 detail the cure of blaOXA-51-

like genes from *A. baumannii* isolates and the positive PCR results for the genes from plasmid DNA extractions (Pournaras et al., 2006). The second hypothesis is that the blaOXA-51-like genes may be found on plasmids and that they might be transmitted to *Escherichia coli*. An *A. baumannii* has a G+C content of 38.4–39.9 percent, indicating that blaOXA-51-like genes are present inherently (Towner and Bergogne-Berezin., 1996).

The role played by *A. baumannii's* OXA-51-like enzymes in carbapenem resistance is still unclear. Only OXA-51 and OXA-69 have been looked at for their kinetic characteristics out of the 73 different types (Fournier et al., 2005). The hydrolytic characteristics of the two enzymes were not comparable in terms of their ability to against carbapenems. OXA-69 has a low ability for meropenem and imipenem but was still susceptible to weakly hydrolyze both of these antibiotics. On the other hand, OXA-51 was significantly more sensitive to imipenem, but was still only susceptible to hydrolyze imipenem weakly, with no detectable meropenem hydrolysis. Further kinetic investigations of OXA-51-like enzymes are necessary in order to identify the level of variance in their ability against carbapenems within this enzyme group (Evans et al., 2008).

1.6.2.8 OXA-58 Cluster

It was discovered in 2003 that a carbapenem-resistant *A. baumannii* clinical isolate had been isolated from a French hospital. *A. baumannii* OXA-58, the first member of a fourth category of OXA-type beta-lactamasemases, was shown to be responsible for the resistance. Afterwards, three more enzyme variations were identified; OXA-96 was discovered in a Singaporean isolate from 1996. A hospital in Tunisia was discovered to encode OXA-97, whereas six isolates consecutively isolated from a patient in Germany were encoded by OXA-164. The OXA-58-like group consists of these four enzymes (Evans et al., 2013).

It was discovered that the kinetic features of OXA-58 were comparable to those of other *A. baumannii* class D OXA-type beta-lactamasemases with carbapenemase ability. As determined by kinetic studies, the enzyme exhibited weak activity against penicillins and carbapenems and variable activity against cephalosporins, with cephalothin and cefpirome hydrolysis identified, but not cefotaxime or ceftazidime (Poirel et al., 2005).

1.6.2.9 OXA-143 cluster

One of the most recently discovered OXA type beta-lactamase, OXA-143, has been identified as a fifth class D OXA beta-lactamase. The enzyme was found on a 30 kb plasmid in three Brazilian isolates and had 88 percent amino acid similarity with OXA-40 (Haggins et al., 2009). Homologous recombination is believed to have mobilized the blaOXA-143 gene, which is not linked to any insertion sequences or integrons. At low levels, penicillin and carbapenem hydrolysis can be detected, but no hydrolysis of cephalosporins can be detected except for the hydrolysis of cephalothin by the enzyme. The carbapenem MICs of imipenem and meropenem were elevated from 0.19 to 32 mg L-1 after the blaOXA-143 gene was transferred to the sensitive *A. baumannii* ATCC 19606 strain. Newly discovered enzyme, OXA-182, has 93% amino acid similarity with OXA-143 in 12 clonally related strains from Korea (Kim et al., 2010).

1.6.2.10 Other beta-lactamasemases

The new delhi metallo—lactamase (NDM) has been an increasingly prevalent mechanism for *A. baumannii* resistance to carbapenems in recent years. First, a case of NDM-1 made by *A. baumannii* was found in an Indian patient in 2010 for the first time (Karthikeyan et al., 2010). Frequently, the blaNDM gene is contained inside a mobile ISAba125-bounded composite transposon of 10 kb termed Tn125, This is a characteristic that is often seen on conjugative plasmids (Hamidian and Nigro., 2019). Since then, there has been an increase in the number of cases of CRAB in Europe, Egypt, China, and Japan since the discovery of NDM-1 and NDM-2 as potential triggers. Finally, while it has not been extensively documented, *A. baumannii* carbapenem resistance has been linked to Klebsiella pneumoniae carbapenemase (KPC), a class A enzyme. KPC-positive *Acinetobacter* isolates from Puerto Rico were identified in the research, which included blaKPC2, blaKPC3, blaKPC4 and blaKPC10 (Pogue et al., 2013).

1.6.3 Non-enzymatic mechanisms

Carbapenem resistance has also been attributed to nonenzymatic processes, such as alterations in the outer membrane proteins of the bacterial cell wall (OMPs), multidrug efflux pumps, and change of penicillin-binding protein affinity or expression (Peleg et al., 2008).

1.6.3.1 Changes in OMPs and PBPs

Porins, or outer membrane proteins (OMPs), play a critical role in the antibiotic resistance of *A. baumannii*. Because of this, it is impossible to correctly measure OMP losses. The amounts of OMPs that have been detected have been shown to vary in laboratory research. PBPs have shown a similarly complex picture (Perez et al., 2007).

Investigators in New York City investigating the MDR *A. baumannii* outbreak discovered isolates with decreased 37, 44, and 47-kDa OMP expression as well as elevated class C cephalosporinases in the city's carbapenem-resistant isolates (Quale et al., 2003), While just a limited number of isolates were examined in that publication, OXA or MBL enzymes were not systematically examined. Carbapenem resistance was also found in Madrid isolates due to the loss of 22-kDa and 33-kDa OMPs and the formation of OXA-24 (Bou et al.,2000).

An *A. baumannii* 43-kDa protein recently shown to be a homologue of OprD has been found (in *Pseudomonas aeruginosa*, a well-studied porin commonly linked to imipenem resistance). It is for CarO, a 29-kDa OMP, the channel development of which confers resistance to imipenem in *A. baumannii* has been well described. Reduced expression of PBP-2 in *A. baumannii* may potentially explain its resistance to carbapenems, as was shown in isolates from Seville, Spain. These isolates were notable for their loss of OMPs and production of beta-lactamases, demonstrating the interaction of several mechanisms of resistance to a class of antibiotics (Perez et al., 2007).

1.6.3.2 Efflux pump

Efflux pumps represent a unique feature in drug resistance: a single mechanism producing resistance to many types of antibiotics. Antibiotics, as well as other toxins, may be expelled from the bacterial cell by these multicomponent pumps in a regulated exchange of protons. Different efflux pump families occurring in a broad variety of bacterial species have been identified (Poole., 2005). Efflux pump AdeABC from the resistance-nodulation-cell division family has been extensively studied in *A. baumannii*. A variety of antibiotics, such as erythromycin, trimethoprim, tetracyclines, fluoroquinolones, chloramphenicol and cefotaxime, are pumped via this system (Magnet et al., 2001). Antibiotic resistance to carbapenems may be facilitated by the overexpression of the AdeABC efflux pump (hydrolytic oxacillinases in combination with carbapenems). This pump has a two-step regulator (adeR) and sensor (adeS) system. A single point mutation in the adeR or adeS gene leads to enhanced expression and therefore increased efflux (Perez et al., 2007). One of the multidrug and toxic compound pumps, AbeM, was recently found in *A. baumannii*. It was recently found. Aside from fluoroquinolones, it seems to have a restricted range of antibiotic substrates (Su et al., 2005).

2. Material method

2.1 Study design:

A case-control study in which 80 samples of different *A. baumannii* from different clinics and specimen complaining of the carbapenems resistant which diagnosed by medical microbiologist as a case of carbapenems resistant.

2.2 Sample collection

A total of 80 samples were isolated from (pus, wound swab, catheter, sputum and urine) samples taken from different clinics in Tokat Gazi Osmanpaşa university hospital, and the practical issues were done to obtain the glycerol stock to revive bacteria. These samples were previously determined to be *Acinetobactr baumannii* through the vitek-2 (bioMerieux, France) and were resistant to carbapenems such as meropenem and imipenem.

2.3 Bacterial reviving and staining

A. baumannii isolates were revived in the first stage by culturing them on blood agar and eosin methylene blue (EMB) agar, drawing loops from stocks and culturing them on blood agar and EMB by using the streaking method, then incubating them at $37C^o$ for 24 hours. After incubation, a diagnosis is made. Then did gram stain to be insured that it is coccobacillus and gram negative.

Figure 3: glycerol stocks

2.4 Culturing on Eosin Methylene blue

On EMB agar, *A. baumannii* was cultured, but its colonies were colorless because it could not ferment lactose, an essential component of this medium.

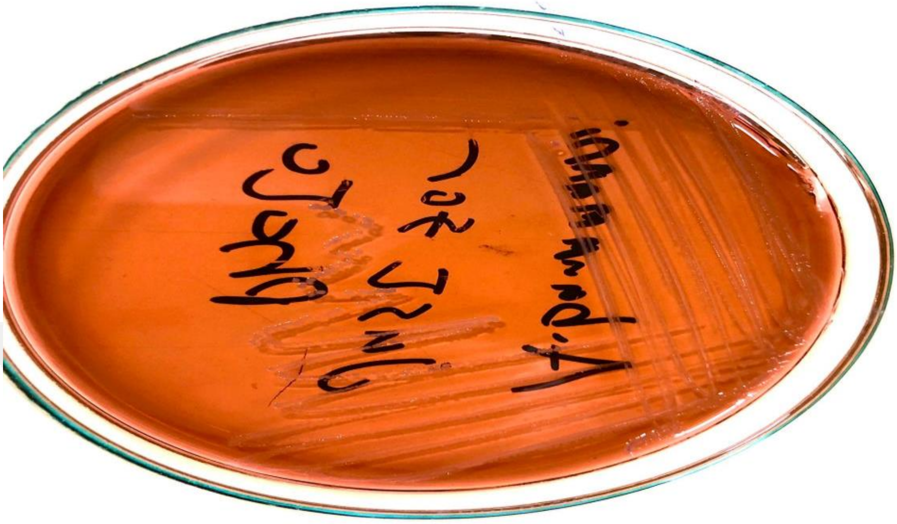

Figure 4: *A. baumannii* on EMB agar

2.5 Culturing on Blood agar

Colonies resemble Enterobacteriaceae on blood agar, appear as smooth round colony.

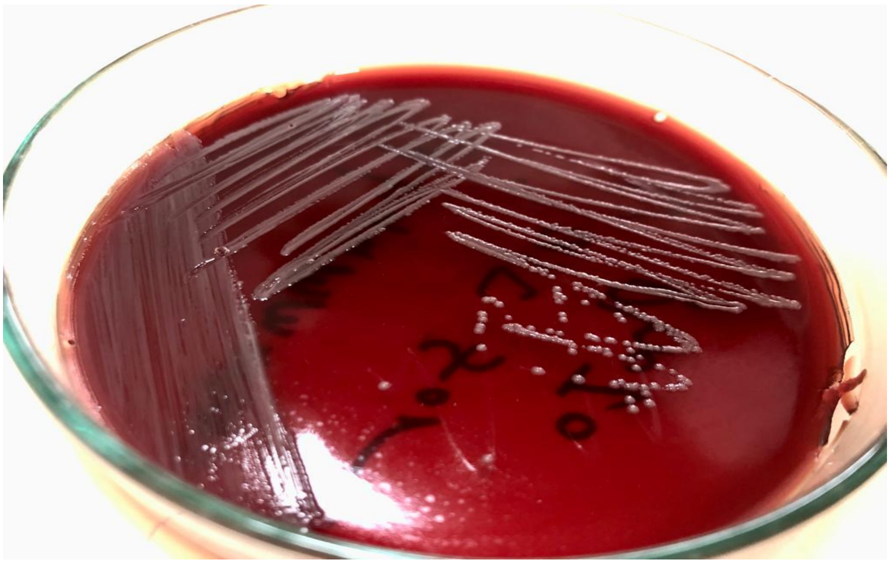

Figure 5: *A. baumannii* on blood agar

2.6 Antibiotic Susceptibility test

The disk diffusion method was used to test *A. baumannii* for carbapenems antibiotic resistance. Placed both meropenem (10μg MEM) and imipenem (10μg IMP) as carbapenem antibiotics onto Mueller-Hinton agar, which was streaked with 0.5 McF of *A. baumannii* suspension, incubated for 24 hours at 37C°. Then measured the inhibition zone to determine if carbapenems were resistant.

Table 1: Zone diameter breakpoint

Zone diameter breakpoints (mm)		
Carbapenems	**S ≥**	**R<**
Meropenem	24	21
Imipenem	21	15

2.7 Preparing Mueller-Hinton agar

We added 38 grams of Mueller-Hinton agar to 1000ml of distilled water and kept the mixture, which we prepared as pH 7.3, for 15 minutes in the autoclave at 121C°, waited for it to cool down to 50C°, and poured on sterile petri dishes.

2.8 Carbapenem-inactivation methods

The Clinical and Laboratory Standards Institute (CLSI) has approved phenotypic methods that clinical microbiology labs can use to find carbapenemase-producing bacteria (Sine et al., 2018). Used to identify the presence of the carbapenems enzyme in *A. baumannii* strains, which is responsible for inactivating carbapenem antibiotics. First, we drew 10μL loopful of *A. baumannii* strains to make a suspension with 400μL of distilled water and immerged meropenem10μg, then incubated at 35°C for 4 hours. We made o.5μCfarland of *Escherichia coli* ATCC suspension and streaked it onto Mueller-Hinton agar, then left it for 3-10 minutes at room temperature to be dry. After 5 hours of incubation of meropenem suspension, we drew meropenem from suspension and poured it on streaked Mueller-Hinton agar with *Escherichia coli* ATCC, then incubated at 37°C for 18–24 hours. By measuring inhibition zone diameter, we decided which strain has the presence of the carbapenems enzyme to inactivate carbapenem antibiotics. the result to be positive for inhibition zone diameters of 0-16mm or the satellite growth of colonies of 16–18 mm, and ≤19mm to be negative (Van der Zwaluwet al., 2015).

Figure 6: Carbapenem-inactivation method disk on streaked *Escherichia coli.*

2.9 DNA Isolation

A. baumannii DNA was isolated by using the boiling method, which is used to get pure DNA from bacterial cells. We cultivated bacteria on blood agar at 37^{o}C for 24 hours. After incubation, we sub-cultured on Muller-Hinton agar at 37^{o}C for 24 hours. Each tube was filled with 500µL of distilled water. We drew a loopful of colony from Mueller-Hinton agar immerged in the tube, then vortexed to dissolve colony completely. Then boiled for 15 minutes in heater set to 100^{o}C to dissolve cells and DNA to be free from cells. After boiling, we centrifuged to separate free DNA from other components at 15.000 rpm for 20 minutes in 4^{o}C of temperature. Then collected supernatant, which included bacterial DNA transferred to the new tube (Al-Amri et al., 2020)

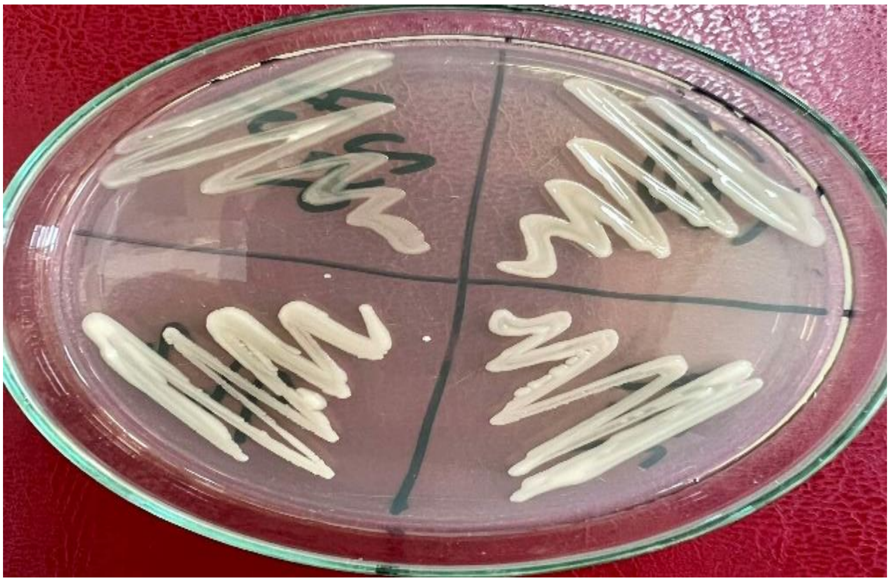

Figure 7: Cultured *A. baumannii* on Muller-Hinton agar to DNA isolation.

2.10 Detection of genes(KPC, NDM, OXA and IMP):

2.10.1 DNA Amplification:

Done by using Polymerase change reaction (Conversional PCR) as following steps: Prepared the master mix by adding (Distilled H_2O) 16.6µl to each Eppendorf tube. added buffer 2.5µL and MgCl2 1.5µL, then mixed with the filled Eppendorf via distilled water. To the filled Eppendorf with mixed distilled water, added 0.3µL of dNTP, 0.8µL of forward (KPC, NDM, OXA-48 or IMP) primer and 0.8µL of reverse primer (KPC, NDM, OXA-48 or IMP), and 0.5µL of Taq polymerase. Following that added 2.0µL of purified DNA for each Eppendorf tube which contain Master mix.

Table 2: primers

Gene	Primer	Sequence 5'-3'	Amplicon size
IMP	**IMP-F**	GGAATAGAGTGGCTTAATTC	678bp
	IMP-R	CCAAACCACTACGTTATC	
OXA-48	**OXA-48-F**	TTG GTG GCA TCG ATT ATC GG	438bp
	OXA-48-R	CTC AGT GCT CTA CAG AAA ACC	
NDM	**NDM-F**	GCA GCT TGT CGG CCA TGC GGG C	782bp
	NDM-R	GGT CGC GAA GCT GAG CAC CGC AT	
KPC	**KPC-F**	TGT CAC TGT ATC GCC GTC	900
	KPC-R	CTC AGT GCT CTA CAG AAA ACC	

2.10.2 Performing the primer protocol in the PCR device:

Step 1. Holding time 1 cycle: at 95C^{o} for 5:00 minutes

Step 2. Cycling: 35 cycles

Denaturation: at 95 C^{o} for 00:45 seconds

Annealing: at 60 C^{o} for 00:45 seconds

Extension: at 72 C^{c} for 1:00 minute

Step 3. Final extension 1 cycle : at 72C^{o} for 8:00 minutes.

2.10.3 Performing of gel electrophoresis:

Prepared the agarose gel by diluted 100ml of 10X TBE buffer solution with 900ml of distilled water to make 1X TBE buffer , and by mixing 1.5 gram of agarose powder in 10ml of diluted TEB buffer solution, then heated in the oven to dissolve the agarose powder in the buffer solution till it disappeared, then added 5 µL of Ethidium bromide to melted gel powder. Poured the liquefied gel in the casting ray then comb inserted into liquefied gel to make wells, and allowed the gel to be solidified at room temperature. After solidified removed caps and comb to reveal loading wells, submerged the gel under TBE buffer in the electrophoresis chamber. Mixed one drop of DNA samples with one drop of loading buffer dyes loaded then into the wells, then loaded 100 DNA ladder. Then started electrophoresis by applying electric field for one hour at 120 volts, separating nucleic acid fragments in the gel on the basis of size. stopped electrophoresis, then exposed the gel to UV light to show bands of the gens.

Figure 8: bands of the genes under UV light

Table 3: apparatus and equipment's

Apparatus and Equipment's	Company
Thermocycler	BIOER
EMB agar	Himedia
Blood agar	Himedia
Mueller-Hinton agar	Himedia
Pippete	Transferpette
Primers	Sentebiolab
Vortex	Velp

Centrifuge	Hettich
Heater	Eppendorf
Electrophoresis power supply	Cleaver
Meropenem10 microgram	LIOFILCHEM
Imipenem10 microgram	Bioanalyse
Normal saline	bioMerieux SA
DNA Ladder	Thermoscientific
AGAROSE	BIOMAX
10XTEB Electrophoresis Buffer	Thermoscientific
Electrophoresis chamber	Fisher scientific

3. Result

3.1 Proportion of the specimens

In this study, a total of 80 carbapenem resistant isolates were tested in the study. These isolates were isolated from various clinical specimens. The majority of the clinical specimens were sputum specimens (n=52, 65%), followed by blood (n=15, 19%) wound specimen (n=7, 9%) urine specimen(n=5, 6%) and sterile body fluid (n=1, 1%).

Table 4: proportion of specimens

Specimens	Frequency	Percent
Sputum	52	65%
Blood	15	19%
Wound	7	9%
Urine	5	6%
Sterile body fluid	1	1%
Total	**80**	**100%**

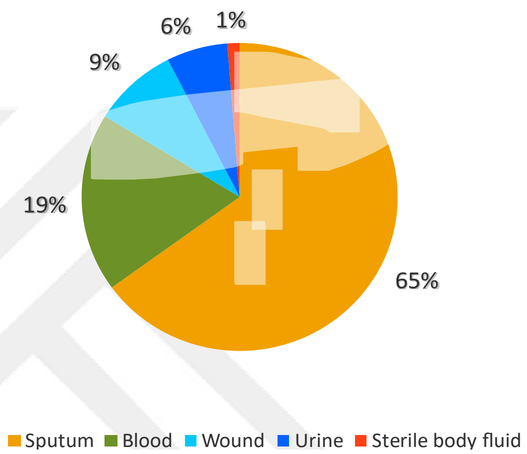

Figure 9: proportion of specimens

3.2 proportion of the clinics

A total 80 samples were collected from 16 various clinics, most of clinics collected were from neurosurgery (n= 14, 17.5%) followed by the department of neurology (n= 10, 12.5%) internal medicine intensive care (n= 10, 12.5%) intensive chest care (n= 9, 11%) orthopedics (n= 6, 7.5%) internal disease (n=8, 10%) pediatric (n= 4, 5%) oncology (n= 4, 5%) general surgery (n= 4, 5%) urology (n= 3 3.8%) Cardiology (n= 2, 2.5%) infection (n= 2, 2.5%) emergency (n= 1, 1.3%) gastroenterology (n= 1, 1.3%) plastic surgery (n= 1, 1.3%) and palliative care service (n= 1, 1.3%).

Table 5: Proportion of clinics

Clinics	Total	Percent
Neurosurgery	14	17.5%
Neurology	10	12.5%
Internal medicine intensive care	10	12.5%
Chest intensive care	9	11%
Internal diseases	8	10.0%
Orthopedics	6	7.5%
Pediatric	4	5%
Oncology	4	5%
General surgery	4	5%
Urology	3	3.8%
Cardiology	2	2.5%
Infection	2	2.5%
Emergency	1	1.3%
Gastroenterology	1	1.3%
Plastic surgery	1	1.3%
Palliative care service	1	1.3%
Total	**80**	**100%**

3.3 Rates of carbapenem resistance using antibiotic susceptibility test

The isolates in this study (n = 80, 100%) were found to be resistant to meropenem and imipenem. Inhibition zone of Imipenem resistant and Meropenem for *A. baumannii* is:

Table 6: Zone diameter breakpoints

Carbapenems	S ≥	R<
Meropenem	24	21
Imipenem	21	15

3.4 Phenotypic detection of carbapenemase enzyme production by using carbapenem-inactivation method

To evaluate the production of carbapenemase in isolates, in our study, we used the carbapenem-inactivation method that was done on Muller-Hinton agar. For the carbapenem-inactivation method, (n = 50, 63%) of the isolates were found to be positive, while (n = 30, 37%) of the isolates were found to be negative.

3.5 Positive rates of carbapenem-resistant genes

In this investigation, 14/38 (33%) of the isolates were determined to be positive for IMP, 11/80 (14%) isolates were detected positive for blaOXA-48, for NDM 9/80 (11%) isolates were determined as positive, and 8/80 (10%) isolates were positive for NDM+KPC and KPC weren't detected without NDM.

Figure 10: Band of the genes

Table 7: Positive rates of (OXA-48, NDM, and KPC) genes

GENES	FREQUENCY	PERCENT
OXA-48	11	14%
NDM	9	11%
NDM+KPC	8	10%
KPC	0	0%
Negative	52	65%
TOTAL	80	100%

Table 8: Positive rates of IMP gene

GENES	FREQUENCY	PERCENT
IMP	11	26%
NDM+KPC+IMP	2	5%
IMP+OXA-48	1	2%
Negative	24	67%
TOTAL	38	100%

4. Discussion:

During the last several decades, *A. baumannii* has evolved as a global nosocomial infection. One of the most successful MDR organisms, it can acquire or upregulate several resistance determinants, making it one of the most effective MDR organisms, able to resist existing antibiotic treatment (Clark et al., 2016). There is now a significant number of these isolates that are carbapenem-resistant *A. baumannii* (CRAB), which is to say that they are pan drug-resistant (PDR) or extensively drug-resistant (XDR) *A. baumannii* (Isler et al., 2019). CRAB can be acquired from patients with a variety of risk factors, including disease severity, prior antibiotic use, invasive procedures such as catheterizations and mechanical ventilation, long duration in the intensive care unit (ICU), previous colonization with *A. baumannii*, and a high work load, according to previous research (Sheng et al., 2010).

In recent years, the growth of carbapenem (imipenem and meropenem) resistance among these bacteria has become a major clinical concern, particularly in poor and medium-income nations (Young et al., 2019). Because these countries do not have access to well-organized antibiotic resistance monitoring systems, inappropriate antibiotic usage will occur both among patients and among those in the healthcare sector (Azimi et al., 2019).

The results of our investigation for the antibiotic susceptibility test showed that all of the samples (n = 80, 100%) were resistant to meropenem and imipenem antibiotics.

In another study done by (Tarafdr et al., 2020) The *A. baumannii* strains evaluated showed a high degree of resistance to imipenem (94%) and meropenem (94%), respectively. MDR strains were found in 98% (49/50) of the *A. baumannii* strains tested. In a study done by (Gomaa et al., 2017) The carbapenem (imipenem and meropenem) resistance rate of *A. baumannii* was 71.4% (40/56) and 39.3% (22/56) of isolates were MBL producers.

These isolates showed MDR or XDR resistance to most of the antibiotic classes that were tested, this may be the result of the extensive use of antimicrobial agents inside hospitals; the simple accessibility and careless administration of these medications outside of hospitals, and that many antibiotics can be bought over-the-counter so that people can treat themselves. Antibiotic resistance has been linked to a higher mortality and illness rate among hospitalized patients, especially in intensive care units (ICUs).

Phenotypic detection of carbapenemase producing isolates using carbapenem-inactivation method. Minimum inhibition concentration MICs do not always represent the generation of carbapenemases since other processes, such as the loss or increase in efflux pump activity, may also generate resistance owing to alterations in chromosomal-located genes, can also produce resistance. To overcome the limited sensitivity of CIM in *Acinetobacter spp*. owing to the low carbapenemase activity and low membrane permeability (Liu et al., 2018).

In our study, the results of the phenotypic detection of CIM of 50/80 (63%) isolates were detected as positive for the carbapenem-inactivation method. In another study done by (Kuchibiro et al., 2021) the results were 16/26 (76.5%).

The first *Pseudomonas aeruginosa* clinical strain that was obtained in 1988 in Japan included the first imipenem-resistant metallo-beta lactamase in the IMP (active on imipenem) group. A multidrug-resistant *A. baumannii* strain isolated from the respiratory tract of a patient in an intensive care unit in Italy was the source of the first report of an IMP-type enzyme in Europe (Ramirez et al., 2020). The IMP and VIM variants confer a high level of carbapenem resistance in *A. baumannii* isolates, as well as resistance to all beta-lactamasems except aztreonam, because of their strong hydrolytic efficiency against these antibiotics (Poirel et al., 2006).

In our study, the IMP gene was detected in 14/38 (33%) of the isolates that carried the blaIMP gene. In another research done by (Szejbach et al., 2013) in Poland on carbapenem resistant *A. baumannii*, it was shown that 10.3% of isolates harboured the blaIMP-like gene.

In 2001, the first OXA-48 like beta-lactamasemase was found in a *Klebsiella pneumoniae* strain that was found in urine from Istanbul, Turkey. During the decade that followed, however, organisms with blaOXA-48-like genes spread quickly across India and a number of countries in Europe, the Middle East, and North Africa, making these areas repositories for isolates that made these enzymes. Even though OXA-48-lactamase and its variations only have low hydrolytic activity against a few carbapenems, when combined with other mechanisms, they may cause high levels of carbapenem resistance (Hemarajata et al., 2015).

In our study, 11(14%) isolates from 80 samples were positive for the OXA-48 gene. Also in another study done by (Tarafdar et al., 2020) the frequencies of blaoxa-48 gene in *A. baumannii* isolates was 92% (46/50). According to other research by (Cheikh et al., 2018), none of the *A. baumannii* isolates had the blaOXA-48 gene. Another study by (Romanin et al., 2019) It was reported that no *A. baumannii* isolates were found to possess the blaOXA-48 gene. These data demonstrated that the prevalence of blaOXA-48 may differ by country.

The NDM enzymes are the most recent MBL enzymes discovered in *A. baumannii*. *A. baumannii* from India and Germany has been shown to have the blaNDM gene. The clinical case shows that NDM-producing bacteria have spread across the Middle East after the recent discovery of NDM-producing isolates in Iraq and the Sultanate of Oman. These isolates shown high levels of resistance to all beta-lactamasem antibiotics, including carbapenems. A total of 68% of the isolates had the blaNDM-1 gene. More than 88% of the blaNDM-1 gene's nucleotide sequences were found to be identical to each other, as well as to those found in other nations (mainly including India, Iran, and

Iraq). (Gomaa et al., 2017). As the blaNDM-1 gene is placed in integrons and some of these integrons are situated on conjugative plasmids, it is horizontally transmissible. (Poirel et al., 2011).

In our result, the PCR was conducted to detect blaNDM gene in *A. baumannii* isolates using specific primers was NDM in 9/80 (11%) of the isolates carried blaNDM gene and 8/80 (10%) of the isolates was NDM+KPC. Also in another study done by (Gomaa et al., 2017) in the positive MBL isolates, the MBL-encoding gene *bla*$_{\text{NDM-1}}$ was identified in 13/22 (59.1%) of the MBL-producing isolates. In another study done by (Boral et al., 2019) None of the 172 isolates showed the blaNDM gene.

Klebsiella pneumonia carbapenemase (KPC) producers are prevalent all over the globe, which is of great significance. Gram-negative KPC producers may be resistant to all beta-lactamases except aztreonam. *A. baumannii* and even other Gram-negative bacilli may pick up these carbapenemases, which are found in transferable genetic elements. Hence, identifying *A. baumannii* antibiotic-resistant strains by identifying the organisms that produce carbapenemase in healthcare facilities might be useful (Azimi et al., 2015).

The blaKPC gene spread rapidly among Klebsiella strains and other bacteria, and a significant proportion of multidrug-resistant isolates were discovered to have it. Unfortunately, bacteria that make KPC enzymes are often resistant to multiple drugs. This makes antibiotic treatment options very limited. patients that are infected with KPC bacteria have a high mortality rate. The rapid dissemination of the KPC gene may be attributable to the plasmid-borne transposon Tn4401. (Ramirez et al., 2020).

Our PCR results revealed that KPC was not detected alone in isolates but was detected in combination with NDM positive isolates, KPC+NDM in 8/80 (10%). In another study done by (Azimi et al., 2015), As a result, OXA-23 combined all of the KPC producer 6/63 (9.52%) isolates. In another investigation by (Robledo et al., 2011) The results of 41/291 (14%) isolates were KPC.

In our investigation, we studied the comparison of positive rates between CIM and carbapenemase genes. We didn't find 100% sensitivity by using CIM for *A. baumannii.* in our results, the positive rates of the carbapenem-inactivation method were 26/39 (60%) isolates. Of those, 39 isolates were detected as positive for carbapenemase-producing genes, but the carbapenem-inactivation method gave false negative results in 13/39 (40%) of the isolates. However, Due to the low activity of OXA-type carbapenemases, this method is insensitive to the detection of *A. baumannii* carbapenemase (Simner et al., 2017). Due to OXA carbapenemases, which are weak carbapenemases, and *A. baumannii's* inherent low outer membrane permeability, phenotypic testing for *A. baumannii* has limitations. (Howard et al., 2020).

Table 9: The comparison of positive rates between CIM and carbapenemase genes

GENES	Gene-Positive	CIM-Positive	CIM-Negative
OXA-48	10	3	7
NDM	9	9	0
NDM+KPC	6	4	2
IMP	11	7	4
IMP+KPC+NDM	2	2	0
IMP+OXA-48	1	1	0
Total	39	26	13

In another study The positive rate in the 83 carbapenemase-producing isolates was 6.02% (CIM) (Fan et al., 2020). For 12 *A. baumannii* isolates that made GES-22+OXA-23 (n = 2) and OXA-58 (n = 10) types of carbapenemases, CIM tests with two hours of meropenem incubation gave false negative results. All of these isolates had positive CIM results when they were incubated for four hours instead of two, which showed that they had low levels of carbapenemase. There was no difference between 2 and 4 hours of incubation for *Enterobacteriaceae*. For *A. baumannii* isolates, we suggest that the disc be incubated for at least four hours in order to achieve optimum CIM performance. (Aktaş et al., 2017).

In this study all isolates were resistant to meropenem and imipenem .However the carbapenemase production was not seen both PCR and CIM tests. This results indicating that carbapenem resistance was mediated by different carbapenemase genes or mechanisms. The evaluation of carbapenem resistance determinants is critical for the prevention of health-care-associated infection.

5. Conclusion

1. The highest percentage of carbapenems-resistant strains have been found in sputum specimens and neurosurgery clinics.

3. In contrast to the phenotypic detection of carbapenemase production in isolates, in our study, 50/80 (63%) of the isolates were positive by CIM.

4. In this investigation, the most frequent of the carbapenem resistant genes were 14/38 (33%) of the isolates were determined to be positive for IMP, followed by 11/80 (14%) isolates were detected positive for blaoxa-48, for NDM 9/80 (11%) isolates were determined as positive, and 8/80 (10%) isolates were positive for NDM+KPC and KPC weren't detected without NDM.

6. Recommendation

1. Further studies need to identify all of the carbapenem resistant genes and factors.

2. Further studies are required for new antibiotics to be discovered because the vast majority of *Ainetobacter baumannii* have developed strains resistant to all antibiotics.

3. Reduce the use of antibiotics randomly in hospitals and outside of hospitals and focus on personal protective equipment to avoid passing on resistance genes.

4. The evaluation of carbapenem resistance determinants is critical for the prevention of health-care-associated infection.

7. References

A Evans, B., Hamouda, A., & Gb Amyes, S. (2013). The Rise Of carbapenem-Resistant *A. baumannii*. Current Pharmaceutical Design, 19(2), 223-238.

Afzal-Shah, M., Woodford, N., & Livermore, D. M. (2001). Characterization Of Oxa-25, Oxa-26, And Oxa-27, Molecular Class D Β-Lactamases Associated With Carbapenem Resistance In Clinical Isolates Of *A. baumannii*. Antimicrobial Agents And Chemotherapy, 45(2), 583-588.

Akrami, F., & Namvar, A. E. (2019). *A. baumannii* As Nosocomial Pathogenic Bacteria. Molecular Genetics, Microbiology And Virology, 34(2), 84-96.

Aktaş, E., Malkoçoğlu, G., Otlu, B., Çopur Çiçek, A., Külah, C., Cömert, F., ... & Bulut, M. E. (2017). Evaluation of the carbapenem-inactivation method for detection of carbapenemase-producing gram-negative bacteria in comparison with the RAPIDEC CARBA NP. *Microbial drug resistance*, *23*(4), 457-461.

AlAmri, A. M., AlQurayan, A. M., Sebastian, T., & AlNimr, A. M. (2020). Molecular surveillance of multidrug-resistant *A. baumannii*. *Current Microbiology*, *77*(3), 335-342.

Albrecht, M. A., Griffith, M. E., Murray, C. K., Chung, K. K., Horvath, E. E., Ward, J. A., ... & Wolf, S. E. (2006). Impact Of Acinetobacter Infection On The Mortality Of Burn Patients. Journal Of The American College Of Surgeons, 203(4), 546-550.

Ayoub Moubareck, C., & Hammoudi Halat, D. (2020). Insights Into *A. baumannii*: A Review Of Microbiological, Virulence, And Resistance Traits In A Threatening Nosocomial Pathogen. Antibiotics, 9(3), 119.

Azimi, L., Talebi, M., Pourshafie, M. R., Owlia, P., & Lari, A. R. (2015). Characterization of carbapenemases in extensively drug resistance *A. baumannii* in a burn care center in Iran. *International journal of molecular and cellular medicine*, *4*(1), 46.

Azimi, T., Maham, S., Fallah, F., Azimi, L., & Gholinejad, Z. (2019). Evaluating the antimicrobial resistance patterns among major bacterial pathogens isolated from clinical specimens taken from patients in Mofid Children's Hospital, Tehran, Iran: 2013–2018. *Infection and drug resistance*, *12*, 2089.

Bentancor, L. V., Camacho-Peiro, A., Bozkurt-Guzel, C., Pier, G. B., & Maira-Litrán, T. (2012). Identification Of Ata, A Multifunctional Trimeric Autotransporter Of *A. baumannii*. Journal Of Bacteriology, 194(15), 3950-3960.

Bergogne-Berezin, E., & Towner, K. J. (1996). Acinetobacter Spp. As Nosocomial Pathogens: Microbiological, Clinical, And Epidemiological Features. Clinical Microbiology Reviews, 9(2), 148-165.

Bogaerts, P., Naas, T., Wybo, I., Bauraing, C., Soetens, O., Piérard, D., ... & Glupczynski, Y. (2006). Outbreak Of Infection By Carbapenem-Resistant *A. baumannii* Producing The Carbapenemase Oxa-58 In Belgium. Journal Of Clinical Microbiology, 44(11), 4189-4192.

Boral, B., Unaldi, Ö., Ergin, A., Durmaz, R., & Eser, Ö. K. (2019). A prospective multicenter study on the evaluation of antimicrobial resistance and molecular epidemiology of multidrug-resistant *A. baumannii* infections in intensive care units with clinical and environmental features. *Annals of clinical microbiology and antimicrobials*, *18*(1), 1-9.

Boral, B., Unaldi, Ö., Ergin, A., Durmaz, R., & Eser, Ö. K. (2019). A prospective multicenter study on the evaluation of antimicrobial resistance and molecular epidemiology of multidrug-resistant *A. baumannii* infections in intensive care units with clinical and environmental features. *Annals of clinical microbiology and antimicrobials*, *18*(1), 1-9.

Bou, G., Cerveró, G., Dominguez, M. A., Quereda, C., & Martínez-Beltrán, J. (2000). Characterization Of A Nosocomial Outbreak Caused By A Multiresistant *A. baumannii* Strain With A Carbapenem-Hydrolyzing Enzyme: High-Level Carbapenem Resistance In *A. Baumannii* Is Not Due Solely To The Presence Of Β-Lactamases. Journal Of Clinical Microbiology, 38(9), 3299-3305.

Bou, G., Oliver, A., & Martínez-Beltrán, J. (2000). Oxa-24, A Novel Class D Β-Lactamase With Carbapenemase Activity In An *A. baumannii* Clinical Strain. Antimicrobial Agents And Chemotherapy, 44(6), 1556-1561.

Bou, G., Santillana, E., Sheri, A., Beceiro, A., Sampson, J. M., Kalp, M., ... & Buynak, J. D. (2010). Design, Synthesis, And Crystal Structures Of 6-Alkylidene-2′-Substituted Penicillanic Acid Sulfones As Potent Inhibitors Of *A. baumannii* Oxa-24 Carbapenemase. Journal Of The American Chemical Society, 132(38), 13320-13331.

Cheikh, H. B., Domingues, S., Silveira, E., Kadri, Y., Rosário, N., Mastouri, M., & Da Silva, G. J. (2018). Molecular characterization of carbapenemases of clinical *A. baumannii*–calcoaceticus complex isolates from a University Hospital in Tunisia. *3 Biotech*, *8*(7), 1-8.

Cho, Y. J., Moon, D. C., Jin, J. S., Choi, C. H., Lee, Y. C., & Lee, J. C. (2009). Genetic Basis Of Resistance To Aminoglycosides In Acinetobacter Spp. And Spread Of Arma In *A. baumannii* Sequence Group 1 In Korean Hospitals. Diagnostic Microbiology And Infectious Disease, 64(2), 185-190.

Clark, N. M., Zhanel, G. G., & Lynch Iii, J. P. (2016). Emergence Of Antimicrobial Resistance Among Acinetobacter Species: A Global Threat. Current Opinion In Critical Care, 22(5), 491-499.

Clemmer, K. M., Bonomo, R. A., & Rather, P. N. (2011). Genetic Analysis Of Surface Motility In *A. baumannii*. Microbiology, 157(Pt 9), 2534.

Corbin, B. D., Seeley, E. H., Raab, A., Feldmann, J., Miller, M. R., & Torres, V. J. Ander-® Son Kl, Dattilo Bm, Dunman Pm, Gerads R, Caprioli Rm, Nacken W, Chazin Wj, Skaar Ep. 2008. Metal Chelation And Inhibition Of Bacterial Growth In Tissue Abscesses. Science, 319, 962-965.

Corvec, S., Caroff, N., Espaze, E., Giraudeau, C., Drugeon, H., & Reynaud, A. (2003). Ampc Cephalosporinase Hyperproduction In *A. baumannii* Clinical Strains. Journal Of Antimicrobial Chemotherapy, 52(4), 629-635.

Curiao, T., Morosini, M. I., Ruiz-Garbajosa, P., Robustillo, A., Baquero, F., Coque, T. M., & Cantón, R. (2010). Emergence of bla KPC-3-Tn 4401 a associated with a pKPN3/4-like plasmid within ST384 and ST388 Klebsiella pneumoniae clones in Spain. *Journal of Antimicrobial Chemotherapy*, *65*(8), 1608-1614.

Davis, K. A., Moran, K. A., Mcallister, C. K., & Gray, P. J. (2005). Multidrug-Resistant Acinetobacter Extremity Infections In Soldiers. Emerging Infectious Diseases, 11(8), 1218.

Dexter, C., Murray, G. L., Paulsen, I. T., & Peleg, A. Y. (2015). Community-Acquired *A. baumannii*: Clinical Characteristics, Epidemiology And Pathogenesis. Expert Review Of Anti-Infective Therapy, 13(5), 567-573.

Dijkshoorn, L., Nemec, A., & Seifert, H. (2007). An Increasing Threat In Hospitals: Multidrug-Resistant *A. baumannii*. Nature Reviews Microbiology, 5(12), 939-951.

Eijkelkamp, B. A., Hassan, K. A., Paulsen, I. T., & Brown, M. H. (2011). Investigation Of The Human Pathogen *A. baumannii* Under Iron Limiting Conditions. Bmc Genomics, 12(1), 1-14.

Eijkelkamp, B. A., Stroeher, U. H., Hassan, K. A., Paulsen, I. T., & Brown, M. H. (2014). Comparative Analysis Of Surface-Exposed Virulence Factors Of *A. baumannii*. Bmc Genomics, 15(1), 1-12.

Elhosseiny, N. M., & Attia, A. S. (2018). Acinetobacter: An Emerging Pathogen With A Versatile Secretome. Emerging Microbes & Infections, 7(1), 1-15.

Evans, B. A., Hamouda, A., Towner, K. J., & Amyes, S. G. B. (2008). Oxa-51-Like B-Lactamases And Their Association With Particular Epidemic Lineages Of *A. baumannii*. Clinical Microbiology And Infection, 14(3), 268-275.

Fagon, J. Y., Chastre, J., Domart, Y., Trouillet, J. L., & Gibert, C. (1996). Mortality Due To Ventilator-Associated Pneumonia Or Colonization With *Pseudomonas Or Acinetobacter* Species: Assessment By Quantitative Culture Of Samples Obtained By A Protected Specimen Brush. Clinical Infectious Diseases, 23(3), 538-542.

Falagas, M. E., Karveli, E. A., Kelesidis, I., & Kelesidis, T. (2007). Community-Acquired Acinetobacter Infections. European Journal Of Clinical Microbiology & Infectious Diseases, 26(12), 857-868.

Fattouh, M., El-din, A. N., & Omar, M. A. (2015). Detection of Klebsiella pneumoniae carbapenemase (KPC) producing gram negative superbugs: an emerging cause of multidrug-resistant infections in General Surgery Department of Sohag University Hospital, Egypt. *Int J Curr Microbiol App Sci*, *4*(5), 1-15.

Fitzsimons, T. C., Lewis, J. M., Wright, A., Kleifeld, O., Schittenhelm, R. B., Powell, D., ... & Boyce, J. D. (2018). Identification Of Novel *A. baumannii* Type Vi Secretion System Antibacterial Effector And Immunity Pairs. Infection And Immunity, 86(8), E00297-18.

Fournier, P. E., Vallenet, D., Barbe, V., Audic, S., Ogata, H., Poirel, L., ... & Claverie, J. M. (2006). Comparative Genomics Of Multidrug Resistance In *A. baumannii*. Plos Genetics, 2(1), E7.

Garnacho-Montero, J., & Amaya-Villar, R. (2010). Multiresistant *A. baumannii* Infections: Epidemiology And Management. Current Opinion In Infectious Diseases, 23(4), 332-339.

Glew, R. H., Moellering Jr, R. C., & Kunz, L. J. (1977). Infections With Acinetobacter Calcoaceticus (Herellea Vaginicola): Clinical And Laboratory Studies. Medicine, 56(2), 79-97.

Gomaa, F. A. M., Helal, Z. H., & Khan, M. I. (2017). High Prevalence of bla NDM-1, bla VIM, qacE, and qacEΔ1 genes and their association with decreased susceptibility to antibiotics and common hospital biocides in clinical isolates of *A. baumannii*. *Microorganisms*, *5*(2), 18.

Gradon, J. D., Chapnick, E. K., & Lutwick, L. I. (1992). Infective Endocarditis Of A Native Valve Due To Acinetobacter: Case Report And Review. Clinical Infectious Diseases, 14(5), 1145-1148.

Gradon, J. D., Chapnick, E. K., & Lutwick, L. I. (1992). Infective Endocarditis Of A Native Valve Due To Acinetobacter: Case Report And Review. Clinical Infectious Diseases, 14(5), 1145-1148.

Gu, B., Tong, M., Zhao, W., Liu, G., Ning, M., Pan, S., & Zhao, W. (2007). Prevalence And Characterization Of Class I Integrons Among *Pseudomonas Aeruginosa* And *A. baumannii* Isolates From Patients In Nanjing, China. Journal Of Clinical Microbiology, 45(1), 241-243.

Hamidian, M., & Nigro, S. J. (2019). Emergence, Molecular Mechanisms And Global Spread Of Carbapenem-Resistant *A. baumannii*. Microbial Genomics, 5(10).

Hasan, T., Choi, C. H., & Oh, M. H. (2015). Genes Involved In The Biosynthesis And Transport Of Acinetobactin In *A. baumannii*. Genomics & Informatics, 13(1), 2.

Hemarajata, P., Yang, S., Hindler, J. A., & Humphries, R. M. (2015). Development of a novel real-time PCR assay with high-resolution melt analysis to detect and differentiate OXA-48-like β-lactamases in carbapenem-resistant Enterobacteriaceae. *Antimicrobial Agents and Chemotherapy*, *59*(9), 5574-5580.

Henderson, I. R., Cappello, R., & Nataro, J. P. (2000). Autotransporter Proteins, Evolution And Redefining Protein Secretion. Trends In Microbiology, 8(12), 529-532.

Héritier, C., Poirel, L., Fournier, P. E., Claverie, J. M., Raoult, D., & Nordmann, P. (2005). Characterization Of The Naturally Occurring Oxacillinase Of *A. baumannii*. Antimicrobial Agents And Chemotherapy, 49(10), 4174-4179.

Hidron, A. I., Edwards, J. R., Patel, J., Horan, T. C., Sievert, D. M., Pollock, D. A., & Fridkin, S. K. (2008). Antimicrobial-Resistant Pathogens Associated With Healthcare-Associated Infections: Annual Summary Of Data Reported To The National Healthcare Safety Network At The Centers For Disease Control And Prevention, 2006–2007. Infection Control & Hospital Epidemiology, 29(11), 996-1011.

Higgins, P. G., Dammhayn, C., Hackel, M., & Seifert, H. (2010). Global Spread Of Carbapenem-Resistant *A. baumannii*. Journal Of Antimicrobial Chemotherapy, 65(2), 233-238.

Hood, M. I., & Skaar, E. P. (2012). Nutritional Immunity: Transition Metals At The Pathogen–Host Interface. Nature Reviews Microbiology, 10(8), 525-537.

Houang, E. T., Chu, Y. W., Lo, W. S., Chu, K. Y., & Cheng, A. F. (2003). Epidemiology Of Rifampin Adp-Ribosyltransferase (Arr-2) And Metallo-B-Lactamase (Bla Imp-4) Gene Cassettes In Class 1 Integrons In Acinetobacter Strains Isolated From Blood Cultures In 1997 To 2000. Antimicrobial Agents And Chemotherapy, 47(4), 1382-1390.

Howard, A., O'donoghue, M., Feeney, A., & Sleator, R. D. (2012). *A. baumannii*: An Emerging Opportunistic Pathogen. Virulence, 3(3), 243-250.

Howard, J. C., Creighton, J., Ikram, R., & Werno, A. M. (2020). Comparison of the performance of three variations of the Carbapenem-Inactivation Method (CIM, modified CIM [mCIM] and in-house method (iCIM)) for the detection of carbapenemase-producing Enterobacterales and non-fermenters. *Journal of Global Antimicrobial Resistance*, *21*, 78-82.

Howard, J. C., Creighton, J., Ikram, R., & Werno, A. M. (2020). Comparison of the performance of three variations of the Carbapenem-Inactivation Method (CIM, modified CIM [mCIM] and in-house method (iCIM)) for the detection of carbapenemase-producing Enterobacterales and non-fermenters. *Journal of Global Antimicrobial Resistance*, *21*, 78-82.

Hujer, K. M., Hujer, A. M., Hulten, E. A., Bajaksouzian, S., Adams, J. M., Donskey, C. J., ... & Bonomo, R. A. (2006). Analysis Of Antibiotic Resistance Genes In Multidrug-Resistant Acinetobacter Sp. Isolates From Military And Civilian Patients Treated At The Walter Reed Army Medical Center. Antimicrobial Agents And Chemotherapy, 50(12), 4114-4123.

Husni, R. N., Goldstein, L. S., Arroliga, A. C., Hall, G. S., Fatica, C., Stoller, J. K., & Gordon, S. M. (1999). Risk Factors For An Outbreak Of Multi-Drug-Resistant Acinetobacter Nosocomial Pneumonia Among Intubated Patients. Chest, 115(5), 1378-1382.

Isler, B., Doi, Y., Bonomo, R. A., & Paterson, D. L. (2019). New treatment options against carbapenem-resistant *A. baumannii* infections. Antimicrobial agents and chemotherapy, 63(1), e01110-18.

Jeon, J. H., Lee, J. H., Lee, J. J., Park, K. S., Karim, A. M., Lee, C. R., ... & Lee, S. H. (2015). Structural Basis For Carbapenem-Hydrolyzing Mechanisms Of Carbapenemases Conferring Antibiotic Resistance. International Journal Of Molecular Sciences, 16(5), 9654-9692.

Kanafani, Z., & Kanj, S. (2013). Acinetobacter Infection: Epidemiology, Microbiology, Pathogenesis, Clinical Features, And Diagnosis. Wolters Kluwer, 2, 21-33.

Karageorgopoulos, D. E., & Falagas, M. E. (2008). Current Control And Treatment Of Multidrug-Resistant *A. baumannii* Infections. The Lancet Infectious Diseases, 8(12), 751-762.

Karthikeyan, K., Thirunarayan, M. A., & Krishnan, P. (2010). Coexistence Of Bla Oxa-23 With Bla Ndm-1 And Arma In Clinical Isolates Of *A. baumannii* From India. Journal Of Antimicrobial Chemotherapy, 65(10), 2253-2254.

Kim, C. K., Lee, Y., Lee, H., Woo, G. J., Song, W., Kim, M. N., ... & Chong, Y. (2010). Prevalence And Diversity Of Carbapenemases Among Imipenem-Nonsusceptible Acinetobacter Isolates In Korea: Emergence Of A Novel Oxa-182. Diagnostic Microbiology And Infectious Disease, 68(4), 432-438.

Knapp, S., Wieland, C. W., Florquin, S., Pantophlet, R., Dijkshoorn, L., Tshimbalanga, N., ... & Van Der Poll, T. (2006). Differential Roles Of Cd14 And Toll-Like Receptors 4and 2 In Murine *Acinetobacter Pneumonia*. American Journal Of Respiratory And Critical Care Medicine, 173(1), 122-129.

Korotkov, K. V., Sandkvist, M., & Hol, W. G. (2012). The Type Ii Secretion System: Biogenesis, Molecular Architecture And Mechanism. Nature Reviews Microbiology, 10(5), 336-351.

Kuchibiro, T., Komatsu, M., Yamasaki, K., Nakamura, T., Nishio, H., Kimura, K., ... & Nishi, I. (2021). Comparison of the performance of three carbapenem inactivation methods for the detection of carbapenemase-producing Gram-negative bacilli. *Journal of Infection and Chemotherapy*, *27*(11), 1634-1638.

Lee, C. R., Lee, J. H., Park, M., Park, K. S., Bae, I. K., Kim, Y. B., ... & Lee, S. H. (2017). Biology Of *A. baumannii*: Pathogenesis, Antibiotic Resistance Mechanisms, And Prospective Treatment Options. Frontiers In Cellular And Infection Microbiology, 55.

Lee, J. S., Choi, C. H., Kim, J. W., & Lee, J. C. (2010). *A. baumannii* Outer Membrane Protein A Induces Dendritic Cell Death Through Mitochondrial Targeting. The Journal Of Microbiology, 48(3), 387-392.

Lee, K., Yum, J. H., Yong, D., Lee, H. M., Kim, H. D., Docquier, J. D., ... & Chong, Y. (2005). Novel Acquired Metallo-B-Lactamase Gene, Bla Sim-1, In A Class 1 Integron From *A. baumannii* Clinical Isolates From Korea. Antimicrobial Agents And Chemotherapy, 49(11), 4485-4491.

Lin, M. F., & Lan, C. Y. (2014). Antimicrobial Resistance In *A. baumannii*: From Bench To Bedside. World Journal Of Clinical Cases: Wjcc, 2(12), 787.

Liu, M., Song, Q., Wu, L., Li, M., Chen, Z., Kang, M., & Xie, Y. (2018). Triton X-100 and increased volume of test bacteria in the carbapenem inactivation method enhanced the detection of carbapenemase-producing *A. baumannii* complex isolates. *Journal of Clinical Microbiology*, *56*(3), e01982-17.

Livermore, D. M. (2009). Doripenem: Antimicrobial Profile And Clinical Potential. Diagnostic Microbiology And Infectious Disease, 63(4), 455-458.

Loehfelm, T. W., Luke, N. R., & Campagnari, A. A. (2008). Identification And Characterization Of An *A. baumannii* Biofilm-Associated Protein. Journal Of Bacteriology, 190(3), 1036-1044.

Luke, N. R., Sauberan, S. L., Russo, T. A., Beanan, J. M., Olson, R., Loehfelm, T. W., ... & Campagnari, A. A. (2010). Identification And Characterization Of A Glycosyltransferase Involved In *A. baumannii* Lipopolysaccharide Core Biosynthesis. Infection And Immunity, 78(5), 2017-2023.

Magnet, S., Courvalin, P., & Lambert, T. (2001). Resistance-Nodulation-Cell Division-Type Efflux Pump Involved In Aminoglycoside Resistance In *A. baumannii* Strain Bm4454. Antimicrobial Agents And Chemotherapy, 45(12), 3375-3380.

Manchanda, V., Sanchaita, S., & Singh, N. P. (2010). Multidrug Resistant Acinetobacter. Journal Of Global Infectious Diseases, 2(3), 291.

Maragakis, L. L., Cosgrove, S. E., Song, X., Kim, D., Rosenbaum, P., Ciesla, N., ... & Perl, T. M. (2004). An Outbreak Of Multidrug-Resistant *A. baumannii* Associated With Pulsatile Lavage Wound Treatment. Jama, 292(24), 3006-3011.

Martínez-Martínez, L. (2008). Extended-Spectrum Β-Lactamases And The Permeability Barrier. Clinical Microbiology And Infection, 14, 82-89.

Mcconnell, M. J., Actis, L., & Pachón, J. (2013). *A. baumannii*: Human Infections, Factors Contributing To Pathogenesis And Animal Models. Fems Microbiology Reviews, 37(2), 130-155.

Morris, F. C., Dexter, C., Kostoulias, X., Uddin, M. I., & Peleg, A. Y. (2019). The Mechanisms Of Disease Caused By *A. baumannii*. Frontiers In Microbiology, 10, 1601.

Nordmann, P. (2004). *A. baumannii*, Le Pathogène Nosocomial Par Excellence. Pathologie Biologie, 6(52), 301-303.

Onarheim, H., Høivik, T., Harthug, S., Digranes, A., Mylvaganam, H., & Vindenes, H. A. (2000). Utbrudd Av Infeksjon Med Multiresistent *A. baumannii*. Tidsskrift For Den Norske Legeforening.

Paton, R., Miles, R. S., Hood, J., & Amyes, S. G. B. (1993). Ari 1: B-Lactamase-Mediated Imipenem Resistance In *A. baumannii*. International Journal Of Antimicrobial Agents, 2(2), 81-87.

Peleg, A. Y., Seifert, H., & Paterson, D. L. (2008). *A. baumannii*: Emergence Of A Successful Pathogen. Clinical Microbiology Reviews, 21(3), 538-582.

Perez, F., Hujer, A. M., Hujer, K. M., Decker, B. K., Rather, P. N., & Bonomo, R. A. (2007). Global Challenge Of Multidrug-Resistant *A. baumannii*. Antimicrobial Agents And Chemotherapy, 51(10), 3471-3484.

Pogue, J. M., Mann, T., Barber, K. E., & Kaye, K. S. (2013). Carbapenem-Resistant *A. baumannii*: Epidemiology, Surveillance And Management. Expert Review Of Anti-Infective Therapy, 11(4), 383-393.

Poirel, L., & Nordmann, P. (2006). Carbapenem resistance in *A. baumannii*: mechanisms and epidemiology. *Clinical Microbiology and Infection*, *12*(9), 826-836.

Poirel, L., Bonnin, R. A., & Nordmann, P. (2011). Genetic basis of antibiotic resistance in pathogenic Acinetobacter species. *IUBMB life*, *63*(12), 1061-1067.

Poirel, L., Cabanne, L., Vahaboglu, H., & Nordmann, P. (2005). Genetic Environment And Expression Of The Extended-Spectrum B-Lactamase Bla Per-1 Gene In Gram-Negative Bacteria. Antimicrobial Agents And Chemotherapy, 49(5), 1708-1713.

Poirel, L., Marqué, S., Héritier, C., Segonds, C., Chabanon, G., & Nordmann, P. (2005). Oxa-58, A Novel Class D B-Lactamase Involved In Resistance To Carbapenems In *A. baumannii*. Antimicrobial Agents And Chemotherapy, 49(1), 202-208.

Poirel, L., Naas, T., & Nordmann, P. (2010). Diversity, Epidemiology, And Genetics Of Class D B-Lactamases. Antimicrobial Agents And Chemotherapy, 54(1), 24-38.

Poole, K. (2005). Efflux-Mediated Antimicrobial Resistance. Journal Of Antimicrobial Chemotherapy, 56(1), 20-51.

Pournaras, S., Markogiannakis, A., Ikonomidis, A., Kondyli, L., Bethimouti, K., Maniatis, A. N., ... & Tsakris, A. (2006). Outbreak Of Multiple Clones Of Imipenem-Resistant *A. baumannii* Isolates Expressing Oxa-58 Carbapenemase In An Intensive Care Unit. Journal Of Antimicrobial Chemotherapy, 57(3), 557-561.

Quale, J., Bratu, S., Landman, D., & Heddurshetti, R. (2003). Molecular Epidemiology And Mechanisms Of Carbapenem Resistance In *A. baumannii* Endemic In New York City. Clinical Infectious Diseases, 37(2), 214-220.

Queenan, A. M., & Bush, K. (2007). Carbapenemases: The Versatile B-Lactamases. Clinical Microbiology Reviews, 20(3), 440-458.

Raffaele, Z., Maria, G., Maria, T., & Francesco, R. (2011). Genome Organization Of Epidemic *A. baumannii* Strains. Bmc Microbiology.

Ramadan, R. A., Gebriel, M. G., Kadry, H. M., & Mosallem, A. (2018). Carbapenem-resistant *A. baumannii* and *Pseudomonas aeruginosa*: characterization of carbapenemase genes and E-test evaluation of colistin-based combinations. *Infection and drug resistance*, *11*, 1261–1269.

Ramirez, M. S., Bonomo, R. A., & Tolmasky, M. E. (2020). Carbapenemases: Transforming *A. baumannii* into a yet more dangerous menace. *Biomolecules*, *10*(5), 720.

Richards, A. M., Abu Kwaik, Y., & Lamont, R. J. (2015). Code Blue: A Cinetobacter Baumannii, A Nosocomial Pathogen With A Role In The Oral Cavity. Molecular Oral Microbiology, 30(1), 2-15.

Romanin, P., Palermo, R. L., Cavalini, J. F., Favaro, L. D. S., De Paula-Petroli, S. B., Fernandes, E. V., ... & Yamauchi, L. M. (2019). Multidrug-and extensively drug-resistant *A. baumannii* in a tertiary hospital from Brazil: The importance of carbapenemase encoding genes and epidemic clonal complexes in a 10-year study. *Microbial Drug Resistance*, *25*(9), 1365-1373.

Ruiz, M., Marti, S., Fernandez-Cuenca, F., Pascual, A., & Vila, J. (2007). High Prevalence Of Carbapenem-Hydrolysing Oxacillinases In Epidemiologically Related And Unrelated *A. baumannii* Clinical Isolates In Spain. Clinical Microbiology And Infection, 13(12), 1192-1198.

Rumbo, C., Tomás, M., Fernandez Moreira, E., Soares, N. C., Carvajal, M., Santillana, E., ... & Bou, G. (2014). The *A. baumannii* Omp33-36 Porin Is A Virulence Factor That Induces Apoptosis And Modulates Autophagy In Human Cells. Infection And Immunity, 82(11), 4666-4680.

Sandkvist, M. (2001). Type Ii Secretion And Pathogenesis. Infection And Immunity, 69(6), 3523-3535.

Santillana, E., Beceiro, A., Bou, G., & Romero, A. (2007). Crystal Structure Of The Carbapenemase Oxa-24 Reveals Insights Into The Mechanism Of Carbapenem Hydrolysis. Proceedings Of The National Academy Of Sciences, 104(13), 5354-5359.

Scaife, W., Young, H. K., Paton, R. H., & Amyes, S. G. (1995). Transferable Imipenem-Resistance In Acinetobacter Species From A Clinical Source. Journal Of Antimicrobial Chemotherapy, 36(3), 585-586.

Sharma, R., Goda, R., Borkar, S. A., Katiyar, V., Agarwal, S., Kumar, A., ... & Kale, S. S. (2019). Outcome Following Postneurosurgical Acinetobacter Meningitis: An Institutional Experience Of 72 Cases. Neurosurgical Focus, 47(2), E8.

Sheng, W. H., Liao, C. H., Lauderdale, T. L., Ko, W. C., Chen, Y. S., Liu, J. W., ... & Chang, S. C. (2010). A multicenter study of risk factors and outcome of hospitalized patients with infections due to carbapenem-resistant *A. baumannii*. International Journal of Infectious Diseases, 14(9), e764-e769.

Shlaes, D. M., & Bradford, P. A. (2018). Antibiotics—From There To Where?: How The Antibiotic Miracle Is Threatened By Resistance And A Broken Market And What We Can Do About It. Pathogens & Immunity, 3(1), 19.

Shneider, M. M., Buth, S. A., Ho, B. T., Basler, M., Mekalanos, J. J., & Leiman, P. G. (2013). Paar-Repeat Proteins Sharpen And Diversify The Type Vi Secretion System Spike. Nature, 500(7462), 350-353.

Siegman-Igra, Y., Bar-Yosef, S., Gorea, A., & Avram, J. (1993). Nosocomial Acinetobacter Meningitis Secondary To Invasive Procedures: Report Of 25 Cases And Review. Clinical Infectious Diseases, 17(5), 843-849.

Simner, P. J., Johnson, J. K., Brasso, W. B., Anderson, K., Lonsway, D. R., Pierce, V. M., ... & Roe-Carpenter, D. E. (2018). Multicenter evaluation of the modified carbapenem inactivation method and the Carba NP for detection of carbapenemase-producing *Pseudomonas aeruginosa* and *A. baumannii*. *Journal of clinical microbiology*, *56*(1), e01369-17.

Simner, P. J., Opene, B. N., Chambers, K. K., Naumann, M. E., Carroll, K. C., & Tamma, P. D. (2017). Carbapenemase detection among carbapenem-resistant glucose-nonfermenting Gram-negative bacilli. *Journal of clinical microbiology*, *55*(9), 2858-2864.

Su, X. Z., Chen, J., Mizushima, T., Kuroda, T., & Tsuchiya, T. (2005). Abem, An H+-Coupled *A. baumannii* Multidrug Efflux Pump Belonging To The Mate Family Of Transporters. Antimicrobial Agents And Chemotherapy, 49(10), 4362-4364.

Szejbach, A., Mikucka, A., Bogiel, T., & Gospodarek, E. (2013). Usefulness of phenotypic and genotypic methods for metallo-beta-lactamases detection in carbapenem-resistant *A. baumannii* strains. *Medical science monitor basic research*, *19*, 32.

Tarafdar, F., Jafari, B., & Azimi, T. (2020). Evaluating the antimicrobial resistance patterns and molecular frequency of blaoxa-48 and blaGES-2 genes in *Pseudomonas* *aeruginosa* and *A. baumannii* strains isolated from burn wound infection in Tehran, Iran. *New Microbes and New Infections*, *37*, 100686.

Tomás, M. D. M., Beceiro, A., Pérez, A., Velasco, D., Moure, R., Villanueva, R., ... & Bou, G. (2005). Cloning And Functional Analysis Of The Gene Encoding The 33-To 36-Kilodalton Outer Membrane Protein Associated With Carbapenem Resistance In *A. baumannii*. Antimicrobial Agents And Chemotherapy, 49(12), 5172-5175.

Traglia, G. M., Chua, K., Centrón, D., Tolmasky, M. E., & Ramírez, M. S. (2014). Whole-Genome Sequence Analysis Of The Naturally Competent *A. baumannii* Clinical Isolate A118. Genome Biology And Evolution, 6(9), 2235-2239.

Turton, J. F., Ward, M. E., Woodford, N., Kaufmann, M. E., Pike, R., Livermore, D. M., & Pitt, T. L. (2006). The Role Of Is Aba1 In Expression Of Oxa Carbapenemase Genes In *A. baumannii*. Fems Microbiology Letters, 258(1), 72-77.

Uechi, K., Tada, T., Shimada, K., Kuwahara-Arai, K., Arakaki, M., Tome, T., ... & Fujita, J. (2017). A modified carbapenem inactivation method, CIMTris, for carbapenemase production in *Acinetobacter* and *Pseudomonas* species. Journal of Clinical Microbiology, 55(12), 3405-3410.

Valencia, R., Arroyo, L. A., Conde, M., Aldana, J. M., Torres, M. J., Fernández-Cuenca, F., ... & Aznar, J. (2009). Nosocomial Outbreak Of Infection With Pan–Drug-Resistant *A. baumannii* In A Tertiary Care University Hospital. Infection Control & Hospital Epidemiology, 30(3), 257-263.

van der Zwaluw, K., de Haan, A., Pluister, G. N., Bootsma, H. J., de Neeling, A. J., & Schouls, L. M. (2015). The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. PloS one, 10(3), e0123690.

van der Zwaluw, K., de Haan, A., Pluister, G. N., Bootsma, H. J., de Neeling, A. J., & Schouls, L. M. (2015). The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. *PloS one*, *10*(3), e0123690.

Waack, U., Warnock, M., Yee, A., Huttinger, Z., Smith, S., Kumar, A., ... & Sandkvist, M. (2018). Cpaa Is A Glycan-Specific Adamalysin-Like Protease Secreted By *A. baumannii* That Inactivates Coagulation Factor Xii. Mbio, 9(6), E01606-18.

Walsh, T. R., Toleman, M. A., Poirel, L., & Nordmann, P. (2005). Metallo-Β-Lactamases: The Quiet Before The Storm?. Clinical Microbiology Reviews, 18(2), 306-325.

Wang, H., Guo, P., Sun, H., Wang, H., Yang, Q., Chen, M., ... & Zhu, Y. (2007). Molecular Epidemiology Of Clinical Isolates Of Carbapenem-Resistant Acinetobacter Spp. From Chinese Hospitals. Antimicrobial Agents And Chemotherapy, 51(11), 4022-4028.

Weber, B. S., Kinsella, R. L., Harding, C. M., & Feldman, M. F. (2017). The Secrets Of Acinetobacter Secretion. Trends In Microbiology, 25(7), 532-545.

Young, K., Painter, R. E., Raghoobar, S. L., Hairston, N. N., Racine, F., Wisniewski, D., ... & Motyl, M. R. (2019). In vitro studies evaluating the activity of imipenem in combination with relebactam against *Pseudomonas aeruginosa*. *BMC microbiology*, *19*(1), 1-14.

Zhanel, G. G., Wiebe, R., Dilay, L., Thomson, K., Rubinstein, E., Hoban, D. J., ... & Karlowsky, J. A. (2007). Comparative Review Of The Carbapenems. Drugs, 67(7), 1027-1052.