ANAS ALMASALMEH

INVESTIGATION OF THE COLISTIN RESISTANCE OF ACINETOBACTER BY BROTH MICRODILUTION METHOD

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DEPARTMENT OF MEDICAL MICROBIOLOGY

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Approval

We certify that we have read the thesis submitted by **ANAS M. JAMAL AL MASALMEH** titled **"INVESTIGATION OF THE COLISTIN RESISTANCE OF *ACINETOBACTER SPP.* BY BROTH MICRODILUTION METHOD**" and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Medical and Clinical Microbiology.

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Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

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First and foremost, we must give thanks to Allah, the Almighty, who is the source of our sustenance and direction. To God be the glory, I would like to express my gratitude for being able to complete my thesis research and writing. Throughout my life, and even more so during the course of my research, His unfailing grace and mercy have been a constant companion.

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ANAS M.JAMAL AL MASALMEH

Abstract

Investıgation Of The Colistın Resistance Of *Acinetobacter spp*. By Broth Microdilution Method

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Acinetobacter is Gram-negative bacteria's resistance to antibiotics has risen sharply since the 1970s, making it a global concern. Antibiotic resistance could become a global catastrophe if we run out of options for treating specific microorganisms, such as those that produce hospital-acquired infections, but with the ability to transmit throughout community, signaling that antibiotic resistance might become a major disaster. This study was aimed to see the resistance of Acinetobacter by determinde the MIC (Micro Inhabitory concentration) of *Colistin* on Acinetobacter Species Strains that isolated from various samples from different department in Near East Hospital,out of 50 samples there was n=1 (2%) is resistance to colistin . For our study we secured the main two matteral Colistin sulphate (C2700000) 25 mg (the brand is Merk -Sigma-Aldrich).And Mueller Hinton Broth2 (90922), Cation-Adjusted (M-H 2 Broth; Mueller Hinton II Broth) brand is (Merk -Sigma-Aldrich) to do Colistin Susceptibility Tests on Acinetobacter spp by using Broth Dilution Method (MIC method),so We collected the samples from the Near East University laboratories, North Cyprus

Keywords: *Acinetobacter ,Colistin ,resistance ,Micro Inhabitory concenteration.*

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List of Abbreviations

%: Percent sign

°C: Celsius

A. baumannii: *Acinetobacter baumannii*

Acb: *Acinetobacter calcoaceticus, Acinetobacter baumannii*

AK: Amikacin

AMR *:* *Antimicrobial resistance*
AR *:* *Antibiotic resistance*
CDC: Centers for Disease Control and Prevention

COL: Colistin

DNA: Deoxyribonucleic acid

EMB: Eosine Methilene Blue

EPS: extracellular polymeric substances

ESBL: Extended-Spectrum Beta-Lactamase

ESBLs: Extended-Spectrum Beta-Lactamases

ESKAPE: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae, Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Enterobacter species*

et al.: And others

EUCAST: The European Committee for Antimicrobial Susceptibility Testing

g: gram

G: Guanine

hr: hour

ICU: Intensive care unit

IV: Intravenous

mcg /µg: Microgram

MDR: Multi-Drug Resistant

MDRAB: Multi-Drug Resistant *Acinetobacter baumannii*

mg/kg: milligram per kilogram

mg: milligram

CAMH: Cation Mueller-Hinton

MIC: Minimum inhibitory concentration

mL: Milliliter

mm: Millimeter

n: Number

nm: nanometer

No: Number

OMP: Outer membrane protein

OmpA: Outer membrane protein A

OMV: Outer membrane vesicles

OXA: Oxacillinase

PBPs: penicillin-binding proteins

PCR: Polymerase chain reaction

PDR: Pan-Drug Resistant

QC: Quality control

QS: Quorum Sensing

R: Resistant

RNA: Ribonucleic acid

RND: The resistance-nodulation-cell division

S: Susceptible

SPSS: Statistical Package for the Social Sciences

TRNC: The Turkish Republic of Northern Cyprus

UTI: Urinary tract infection

XDR: Extensively-Drug Resistant

µg/mL: Microgram per Milliliter

CHAPTER I

INTRODUCTION

Acinetobacter spp. is Gram-negative bacteria's resistance to antibiotics has risen sharply since the 1970s, making it a global concern. Antibiotic resistance could become a global catastrophe if we run out of options for treating specific microorganisms, such as those that produce hospital-acquired infections, but with the ability to transmit throughout community, signaling that antibiotic resistance might become a major disaster (El-Sayed Ahmed *et al.* 2020). Resistance to anti-bacterial and anti-fungal medications is known as Antimicrobial Resistance (AMR). It is possible to build up resistance to antibiotics in humans, animals, and crops. The spread of antibiotic-resistant microorganisms among humans, animals, and the natural environment constitutes a threat to global health. Infections become more difficult to cure, raising the risk of disease spread, severe sickness, and death. It is becoming extremely difficult or impossible to cure illnesses because of the development of drug-resistant microorganisms. Public health is at risk from AMR. According to the Centers for Disease Control and Prevention (CDC) humans, animals, and the environment are all affected by AMR. At least 2.8 million people in the United States get infected with antibiotic-resistant bacteria each year, and at least 35,000 of them die (CDC 2021). We would be deprived of the following as a result of discontinuing antibiotic use, Priority one would lose the protection for those with compromised immune systems, such as cancer patients and AIDS patients and recipients of organ transplants and premature babies. Another example is lose the using of stents, diabetic pumps, dialysis, and joint replacement surgery. Lastly, we'd most likely miss out on surgery. In many cases, antibiotics are given as a preventative measure prior to a surgical procedure. So, no heart surgeries, no biopsies of the prostate, and no C-sections. May amputations have been caused by skin infections in some cases without antibiotics. Increases the time spent in the hospital. At least a two-fold increase in mortality is expected. When antibiotics became resistant, we had to utilize more potent, toxic, and expensive drugs with a broader spectrum of action. We would be robbed of the self-confidence with which we go about our daily lives if something like what happened in Covid-19 were to occur, we may be killed by any injury. If we do not get this under control by the year 2050, the global toll is expected to reach 10 million fatalities per year" according to a

British government-funded research known as the Review on Antimicrobial Resistance".

"*Acinetobacter*: an old friend, but a new enemy" (Towner 2009) As one of the most common microorganisms found in the human body, *Acinetobacter spp*. is found all over the world. The *Acinetobacte*r genus has been added to the World Health Organization's "apriority list of antibiotic resistant bacteria" (Chapartegui-González et al. 2021; Tacconelli et al. 2018).List of bacteria for which new antibiotics are urgently necessary is published by the World Health Organization (WHO) Priority 1: CRITICAL (includes *Acinetobacter*, *Pseudomonas*, and several *Enterobacteriaceae*) Available online: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed. *Acinerobater baumannii* (*A. baumannii*) is a Gram-negative opportunistic pathogen is now recognized as a leading source of hospital-acquired infection in the past several years. fatality and illness Estimated annual cases of *Acinetobacter* infections in the United States range about 45,000 per year to a global total of 1 million (Wong et al. 2017).

The aim of this study is investigation of the colistin resistance of *Acinetobacter spp*. by taking 50 samples from the different departments in the Near East university hospital by broth microdilution method, and contributing in small side to help fighting of global threats of AMR.

CHAPTER II

GENERAL INFORMATION

2.1. Taxonomy and History

In 1911, an organism named *Micrococcus calcoaceticus* was discovered by a Dutch microbiologist, Beijerinck. Over the years, similar organisms were described (Beijerinck 1911) , and assigned to 15 genera and species. Brisou and Prevot suggested the taxonomic genus *Acinetobacter* in 1954. It means non-motile in Greek. Brisou and Prevot distinguished non-motile from motile *Achromobacter* species. *Acinetobacter* was not recognized as the name for the genus until Baumann *et al.* found that the previously isolated species belonged to a single genus. These observations led to the subcommittee on taxonomy of Moraxella and associated bacteria officially recognising *Acinetobacter* in 1971. The genus *Acinetobacter* was described in the 1974 edition of Bergey's Handbook of Systematic Bacteriology(Beijerinck 1911). *Acinetobacter calcoaceticus* (*A. calcoaceticus*) and *Acinetobacter lwoffii* (*A. lwoffii)* were listed in the "Approved List of Bacterial Names". So far the genera *Moraxella, Acinetobacte*r, *Psychrobacte*r, and linked bacteria had listed under the *Moraxellaceae* and order *Gammaproteobacteria*. (Bergogne-Bérézin and Towner 1996; ROSSAU et al. 1991).

Acinetobacter taxonomy is difficult to distinguish due to clustering of closely related species. As antimicrobial sensitivity and clinical relevance vary between genomic species, precise *Acinetobacter* species classification is necessary (Bergogne-Bérézin and Towner 1996)

There are around 2,000 *A. baumannii* genomic sequences available. *A. baumannii*'s paralog-collapsed pan-genome size approaches over 12,000 gene sets.(Harding, Hennon, and Feldman 2018). *A. baumannii*, *A,calcoaceticus*, *Acinetobacter haemolyticu*s (*A. haemolyticus*) , *Acinetobacter johnsonii*, *Acinetobacter junii*, and *A. lwoffii* were all named after Bouvet and Grimont (Bouvet and Grimont 1987).In 2019, Vijayakumar *et al.* reviewed 59 *Acinetobacter* species, of which 11 have established names and 15 have tentative descriptions (Vijayakumar, Biswas, and Veeraraghavan 2019). The *A. calcoaceticus–A. baumannii* complex (ACB complex) contains four strictly linked genomic species: *A. calcoa*ceticus (spp. 1), *A. baumannii* (spp. 2), *Acinetobacter pittii* (spp. 3) and *Acinetobacter nosocomialis* (13TU) (Gerner-Smidt

1992). *Acinetobacter seifertii* and *Acinetobacter dijkshoorniae* were recently added to the ACB complex. Thus, the ACB complex includes five human pathogens "*A. baumannii, A. nosocomialis, A. pittii, AAcinetobacter dijkshoorniae*, and *A. seifertti*" and one environmental *Acinetobacter* which is (*A. calcoaceticus*). (Cosgaya et al. 2016; Nemec et al. 2015)

Iraqibacter was given this name because of an increasing populations in *A. baumannii* infections between Iraq and Afghanistan soldiers and troops (CDC 2004). MDR *A. baumannii* has disseminated to civilian hospitals as a result of the repatriation of injured military personnel from conflict areas. (Peleg, Seifert, and Paterson 2008).

Currently, the genus has 65 species with valid published names by Alexandr Nemec.(www.szu.cz/anemec/Classification.pdf)(http://apps.szu.cz/anemec/anemec.htm). New investigation done by Qin *et al*, added two new *Acinetobacter* species to the taxonomic tree: *Acinetobacter rongchengensis* and *Acinetobacter tianfuensis*, as well as declared *Acinetobacter portensis* a synonym of *Acinetobacter pullorum*. also updated taxonomy, and corrected species assignments. Therefore, the genus *Acinetobacter* now has 144 species, 68 identified and 76 nameless. *Acinetobacte*r is a single genus, according to the research. The identification of the 56 taxa brings the total number of *Acinetobacter* species to 144, 68 recognized and 76 unidentified (Qin et al. 2021).

2.2. Epidemiology

Acinetobacter can be found in soil and water samples (Ash, Mauck, and Morgan 2002; Baumann 1968; Choi et al. 2012).The first strain of *Acinetobacter spp.* was isolated from soil and identified as *Micrococcus calcoaceticus* by Beijerinck in 1911(Beijerinck 1911),as well as it was founded in pets, arthropods, and food animals, in addition to humans. It has the ability to colonize wounds, skin, and the gastrointestinal and respiratory tracts(E. Bergogne 1996). Acinetobacter's infections linked to hospital epidemics (Nandi and Arjuna 2017), war (Camp and Tatum 2010; Hashim, Saleh, and Abdulrazaq 2020), natural disasters (Oncül et al. 2002), and tropical climates (Doughari et al. 2011).

Some *Acinetobacter spp.* primarily *A. lwoffii*, *A. johnsonii* and *Acinetobacter radioresistens*, are part of the skin's bacterial flora and are found mainly in moist skin areas. *A. baumannii*, on the other hand, is thought to be isolated from patients and hospital environmental sources, but not from outside hospitals (Eveillard et al. 2013). However, some studies showed surveillances by utilizing molecular approaches to identify *A. baumannii* revealed that this organism can live outside of hospitals (Peleg et al. 2008). *A. baumannii* infections reported that only 4 percent were community acquired, Whereas more than 90 percent were nosocomial (Fournier and Richet 2006). Meanwhile the 1970s, A*cinetobacter* in the hospitals gradually have appeared as major factor of nosocomial infections, as their importance came from the ability of these species to survival and develop resistance to many antimicrobial, *A.baumanii* out of *Acinetobacter spp*. was and still the most important pathogen are causing the outbakes in the intensive care unit (ICU) in the hospitals around the world. (Bergogne-Bérézin and Towner 1996; Towner 2009). Moreover, hospital kitchen food is one of major resources for *Acinetobacter spp*. In the hospital kitchen without good sanitation system (Carvalheira, Silva, and Teixeira 2021).

Recently COVID-19 patients could suffer from additional sever consequences related to A.*baumannii* especially for the patients who use ventilator equipment (Rangel, Chagas, and De-Simone 2021) as *Acinetobacter* consider from the most important organism isolated from those devices and that will increase the load on the health system.

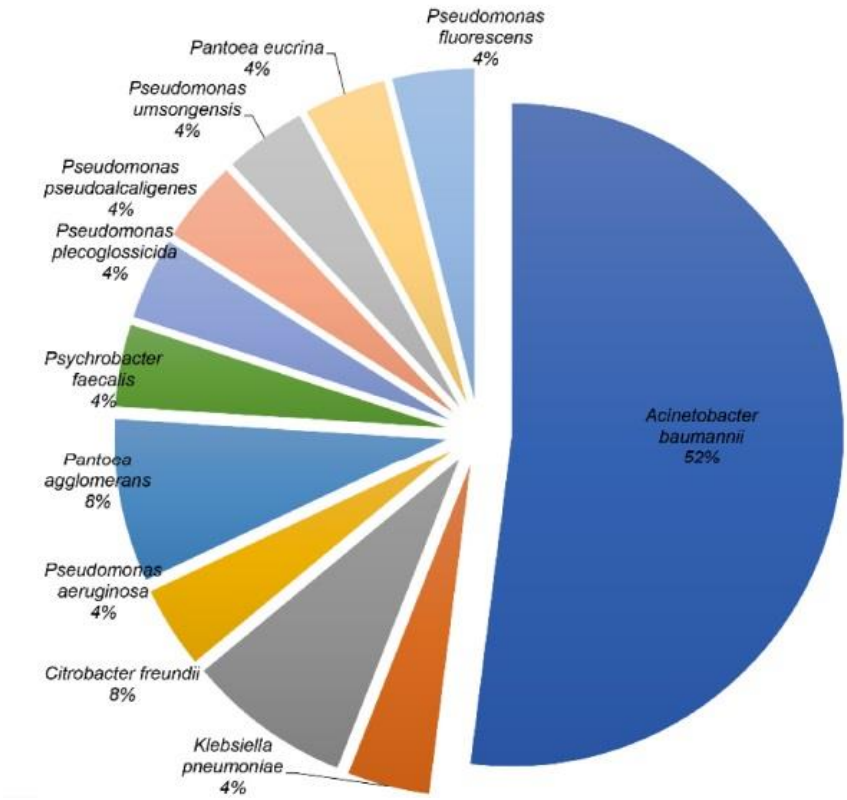


Figure 1. The Distribution Of *A. baumannii* From The Gram Negative Bacteria In The Sixty-Seven Sites (medical devices, surfaces, medical personnel, and patients) from ICU of the Hospital Juarez de Mexico. 2021 (Rangel et al. 2021)

In the time of war and disasters, many reports and studies presented the surveillance of *Acinetobacter spp*. like what had happened in Vietnam, Iraq, and Afghanistan. According to study on the US army showed, that *A. baumannii* was the major pathogen isolated form the wounds of the troops of US army between 2007 and 2008 (Sheppard et al. 2010). Another study done after Marmara earthquake (1999) in Turkey came with same results as A. *baumannii* was produced in (31.2 %) 15 out of 48 from culture specimens which was the biggest percentage between all isolated pathogens.(Oncül et al. 2002).

Distribution of *Acinetobacter* are strongly association with the weather especially there is a prevalence peak in summer or warmer seasons and occurrence trough in winter seasons. (Kritsotakis and Groves-Kozhageldiyeva 2020).

2.3. Pathogenesis and Pathogenic Mechanisms

A. baumannii can be spread by affected patients and their environments, like beds, bed rails, tables, sinks, doors, and medical apparatus. The ventilators, suction devices, and intravascular devices is the most important source of infection (Jung and Park 2015). *A. baumannii* is a low virulence organism except that founded in the immunocompromised patients. The main factors for acquisition *A. baumannii* infection related to immunocompromised patients, prolonged hospitalization, ventilators, intravascular devices, elderly people, previous using of broad-spectrum antibiotic treatment, ICU stay, and enteral feeding. *A. baumannii* considered as a major cause of nosocomial infections, particularly in intensive care units worldwide. The ability of this organism to contaminate hospital surfaces over time has been associated with nosocomial outbreaks (Shimose et al. 2016). It has acquired the ability to infect not only hospital patients but also the general population. In hospitals, it results in a mortality rate of 26%, rising to 43% in ICU (Greene et al. 2016). *A. baumannii* is the leading cause of ventilator-associated pneumonia, accounting for nearly 15% of all hospital-acquired infections, with the highest morbidity and mortality in medical wards and especially in ICU. It accounts for about 50% of total using of antibiotic in ICUs (Demirdal, Sari, and Nemli 2016). Although *A. baumannii* is not a community pathogen in general , however it can cause bronchiolitis and tracheobronchitis in immunocompromised people. It has been linked to community-acquired pneumonia in tropical areas like Asia and Australia, with underlying diseases such as smoking, drinking, diabetes mellitus, and COPD. *A. baumannii* caused bloodstream infections with 10%–15% of patients who had surgical operations due to using foreign objects like intravascular or respiratory catheters, tubes, or cannulas. About 20%-70% of caused *A. baumannii* infections still unknown (Garnacho-Montero et al. 2015). It is responsible for 4% of all meningitis and shunt infections with a mortality rate of 70% in neurosurgery patients (Basri et al. 2015) about 2.1% of ICU-acquired wound infections related to *A.baumannii*; as in battlefield casualties in Afghanistan and Iraq reached to (32%). However, it accounts for 1.6% of total urinary tract infections. It can cause endocarditis, keratitis, and ophthalmitis after contact lens wear and ocular surgery (Peleg et al. 2008).

Acinetobacter's attack requires cell-to-cell connection to create infection. with compared to other microorganisms such as *Pseudomonas aeruginosa*, *Neisseria meningitidis*, and *Campylobacter*. *A.baumanii* has low ability of adhesion to the cells (Asif, Alvi, and Rehman 2018). However, it has a strong hydrophobic capability, which allows it to stick to foreign materials such as plastics used in intravascular devices. The polysaccharide capsule has an important role in inhibiting *Acinetobacter* phagocytosis (Barrie and Gorman 2016). Following adherence, particularly to respiratory epithelial cells. It migrates to the nuclei and mitochondria, where it begins to stimulate the expression of the proapoptotic protein cytochrome c, which causes the host cell to die (Schweppe et al. 2015). A. *baumannii* avoids death via the alternative complement pathway by neutralizing factor H, a critical regulator of the alternative complement pathway, with the help of OmpA (Kim et al. 2016). Secretion of many proteins like proteases, phospholipases, superoxide dismutase, and catalase accelerates the local innate immune reaction and finally leads to tissue destruction.as a result there are many virulence factors contribute in pathogenicity of *Acinetobacter i*n different mechanisms will mentioned in detailed below.

2.4. Virulence Factors

Multiple virulence factors lead to many infections as a result of pathogenicity of *A. baumannii*, and contribute in resistance to many broad spectrum antibiotics. Recently phenotypic and genomic model analyses improve the determination of virulence factors, there are about 16 gene islands effect in pathogenesis of the organism Figure 2 showed the potential and the most important of virulence factor of *A. baumannii* (Moubareck and Halat et al.; 2020).

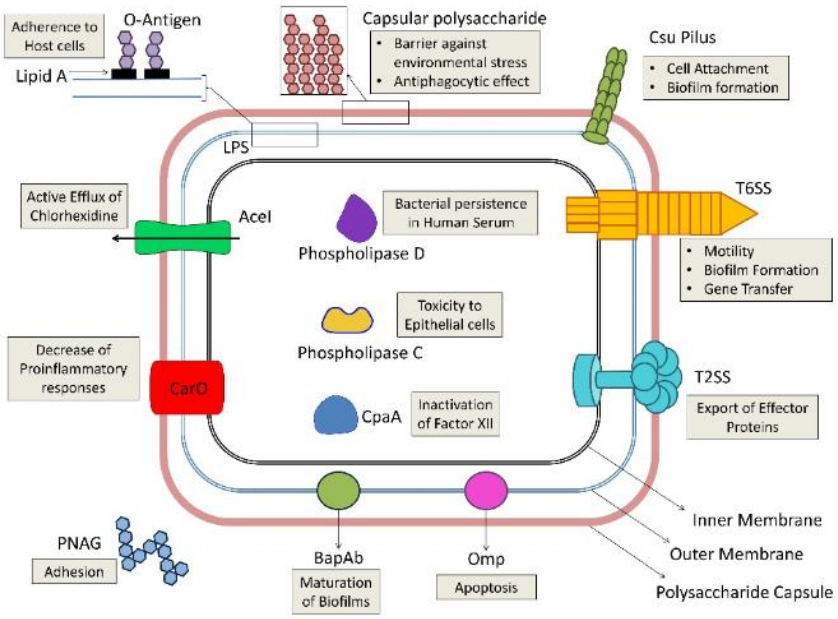


Figure 2. The virulence factors of *A.baumannii (*Moubareck and Halat et al.; 2020)

2.4.1. Outer Membrane Proteins

OmpA, a 38 kDa protein found in *A. baumannii*'s outer membrane, is an important component (Badmasti et al. 2015).They have been linked to antibiotic resistance, pathogenicity, and host cell variation (Uppalapati, Sett, and Pathania 2020). OmpA, is involved in cell invasion and apoptosis. The 38kDa protein is essential for the penetration of tiny solutes. After binding to the host cell membrane, it travels to the mitochondria and nucleus, where it triggers cell death (Choi et al. 2005). Anti-infective defense systems are affected by OmpA, which inhibits the alternative complement pathway while also regulating biochemical activities in bacteria's outer membrane (Kim et al. 2016).

As well as serving as a transporter, OmpA is able to cause host cell death and biofilm formation, as well as disseminate throughout the body and connect with epithelial cells via host fibronectin (Smani, McConnell, and Pachón 2012). The 33- to

36-kDa Omp protein of *A. baumannii* also functions as a water channel and is related with carbapenem antibiotic resistance. Another study found that *A. baumannii* strains lacking Omp 33–36 had abnormal growth rates and a considerably reduced capacity to stick to the host and invade, which suggests that Omp33–36 plays a critical role in *A. baumannii*'s fitness and virulence (Smani, Dominguez-Herrera, and Pachón 2013).

It is common for Gram-negative bacteria to create outer membrane vesicles (OMVs), which help host cells deal with virulence factors. OMVs, which have a diameter ranging from 20 to 200 nm, are mostly composed of lipids, proteins, DNA, and/or RNA, as well as LPS (Jan 2017).Virulence factors are delivered to host cells by OMVs, which also transfer resistance genes between bacteria (Fulsundar, Domingues, and Nielsen 2019).

2.4.2 Biofilm

A. baumannii's multidrug resistance is due to its ability to produce biofilms, one of the most important virulence factors. A biofilm is a collection of bacterial cells adhered to surfaces (Singh et al. 2016). Several host variables, such as growth conditions, cell density, quorum sensing, light, and free iron, influence biofilm formation and regulation (Subhadra et al. 2016). Extracellular polymeric substance (EPS) matrix, which includes exopolysaccharides, nucleic acid, proteins, and macromolecules is used to build the three-dimensional structure of the biofilm (Barraud, Kjelleberg, and Rice 2015).Antibiotic resistance and pathogenicity are both facilitated by the biofilm (Farshadzadeh et al. 2018). 80 percent of all bacterial infections, including cystic fibrosis, otitis media, bloodstream infections, and urinary tract infections, are caused by it (Singh et al. 2016). Glass, polystyrene, and polypropylene are just a few of the non-living materials that *A. baumannii* can develop a biofilm on (Amala Reena, Subramaniyan, and Kanungo 2017). As a virulence factor, the biofilm of *A. baumannii* serves as a barrier against stress in the environment, allowing certain isolates that develop a strong biofilm to persist for lengthy periods of time (Dekic et al. 2017).

In order to coordinate gene transcription, bacteria use the quorum-sensing system to communicate among each other. It releases signals known as "auto inducers," which regulate many physiological functions like virulence factor synthesis and antibiotic resistant development (Subhadra et al. 2016). Tolerating harsh environmental

circumstances like nutritional deficiency and low pH is made possible by the bacterial biofilm building process, which shields bacterial populations from the immune system of the host and so extends the duration of infection (Sharma et al. 2014).

2.4.3 Capsule and Lipopolissacarite

A. baumannii has a polysaccharide capsule attached to its cell wall. Is a significant factor in pathogenicity (Bansal, Harjai, and Chhibber 2014).The pathogenicity of bacteria that cause a variety of diseases, such as pneumonia, cystic fibrosis, tooth rot, and periodontitis, is increased when the capsule is present. Resistance to normal human blood offers a competitive advantage to *A. baumannii* LPS in the *vivo* environment. Pro-inflammatory responses can also be produced in animal models. First-stage colonization could be facilitated by the LPS antigenic O-polysaccharide and pili (Haseley et al. 1997). Besides the LPS, the existence of a capsule around *A. baumannii*'s surface is an important cell structural factor in the bacteria's pathogenicity. As a result of the sugar components in the capsule being repeated and tightly packed, they operate as a barrier against environmental conditions like drying and disinfection as well as immune response reactions like phagocytosis (Singh, Adams, and Brown 2019). This pathogen is able to survive infections since there are more than 100 different varieties *of A. baumannii* capsular polysaccharides (Kenyon and Hall 2013).

2.4.5 Adhesion

The first phase in infection is adhesion to the host's surface, which serves as a foundation for long-term settling and stability. Bacterial instability and non-adhesion to the target surface result in their displacement and eventual disposal (Afshar Payam et al. 2018). Pili and flagella, as well as exopolymeric polymers, are responsible for the bacterial cell's ability to adhere to an external surface (Ishii et al., 2004). Both the shape and function of the pili is taken into consideration while categorizing pili as type I pili and type IV pili which are two examples of pili that can be distinguished on this basis. Pili originate from Gram-negative and Gram-positive bacteria and are linked to twitching motility, genetic recombination, target cell attachment, and biofilm development (Piepenbrink and Sundberg 2016).

2.4.6 Enzymes

The Enzymes and lipolytic activity of *A. baumannii* are an essential virulence factors. Despite the fact that phospholipase D and C are permits *A. baumannii* to survive in human serum and harmful to epithelial cells respectively (Camarena et al. 2010) . Bacterial lipase is primarily secreted in the extracellular fluid. Activity and stability are influenced by pH values, and the enzyme loses its activity *in A. baumannii* if the pH is dropped from 6.5 to 4 in the external medium (Gururaj et al. 2016). Protease enzymes are classified as intracellular or extracellular based on where they are found (Singh et al. 2016). Large proteins are digested by proteases, Proteins are broken down using enzymes that hydrolyze lengthy peptide chains by breaking peptide bonds that hold amino acids together. The incubation duration, temperature, pH, and carbon- and nitrogen-source all have a role in enzyme synthesis (Khusro 2016). Protease is frequently implicated in regulating cell-to-cell communication (King, Pangburn, and McDaniel 2013). There has been a steady accumulation of information on the enzymes of *A. baumannii*, and the enzyme CpaA was recently discovered as a virulence factor that reduces blood cohesion by inactivating factor XII.

2.4.7 Micronutrient Acquisition Systems/Iron, Zinc and Manganese

Due to its propensity to sequester host metals such as iron, manganese, and zinc, *A. baumannii* has survived as a nosocomial pathogen for long periods of time (Tipton and Rather 2017). *A. baumanni*i's various strategies for collecting these sparse nutrients *in vivo*. *A. baumannii* uses five clusters of high-affinity iron-chelating molecules, called siderophores, to capture iron from the surroundings. Catechol-hydroxymate siderophore, Acinetobactin, is the most typically conserved iron-chelating agent (Antunes et al. 2011). Significantly, acinetobactin is essential for virulence (Gaddy et al. 2012) , making the mechanism via which it is synthesized an intriguing target for antibacterial therapy. Fimsbactin A-F(Proschak et al. 2013) and baumannoferrin A-B (Penwell et al. 2015) are also iron scavengers used by *A. baumannii*, although a full genetic and molecular investigation of their biosynthesis and transport machinery is absent. FecA and FecI, two iron transporters and receptors, are also present in the organism, allowing heme consumption (Morris et al. 2019). Virulence can be reduced

by lowering biofilm formation and oxidative stress resistance in models of iron transporter degradation (Ajiboye, Skiebe, and Wilharm 2018). When iron is scarce, *A. baumannii* secretes siderophores, which bind ferric ions and allow the bacterium to collect iron (Katsube, Echols, and Wajima 2019). Siderophores containing Fe^{3+} and heme are obtained by bacteria through particular protein receptors and these receptors are frequently found on the outer membrane of Gram-negative bacteria (Delgado-Valverde et al. 2020). Recent studies on a subject with renal impairment indicated that a new siderophore, cefiderocol (siderophore cephalosporin combination), was well accepted as a therapy for multidrug-resistant strains (Katsube et al. 2017). *A. baumannii* depends on zinc, a structural cofactor for many proteins, for its life. *A. baumannii* employs a zinc acquisition pathway known as ZnuABC (Hood et al. 2012) to counteract calprotectin-mediated nutritional immunity. The ZnuABC transporter ensures intracellular zinc uptake, while the ZigA GTPase is in charge of zinc metabolism in *A. baumannii* (Moore et al. 2014). In addition, *A. baumannii* has a high-capacity zinc scavenging mechanism. Even though *A. baumannii's* mechanisms for dealing with manganese deficiency are poorly understood, a transporter related to the family of resistance-associated macrophage protein (NRAMP) is proposed to enable manganese uptake and promotion in the presence of calprotectin(Juttukonda, Chazin, and Skaar 2016). Studies like this clearly demonstrate the role of micronutrient acquisition systems in *A. baumannii's* pathogenicity and further reinforce the possibility of those systems as novel antibacterial targets.

2.5. Antibiotic Resistance Mechanisms

Three groups can be used to classify the ways antibiotic resistance works. In the first place, resistance can be caused by making the membrane less permeable or making more of the antibiotic escape, which stops the drug from getting to the target. Second, bacteria can change their genes or make changes to their proteins after they've been made. Finally, antibiotics can be broken down or modified to make them less effective (Blair et al. 2015). *Acinetobacter*'s most effective instruments is its ability to change and restructure its genes quickly, as well as to add foreign factors to its DNA that are carried by mobile genetic elements. Penetration sequences are thought to be one of the most important factors in shaping the genomes of bacteria and how they developed (Ayoub Moubareck and Hammoudi Halat 2020). *A. baumannii* can also form biofilms,

which help it stay alive on medical equipment, like ventilation systems in ICUs (Pakharukova et al. 2018). However, the connection between biofilm production and antibiotic resistance is also not clear yet (Yang et al. 2019).

A. baumannii is called "extensive drug resistant" (XDR), if it is resistant to three or more types of antibiotics (penicillins and cephalosporins, including inhibitor combinations, fluoroquinolones, and aminoglycosides, and resistant to carbapenems in most cases). Also, *A. baumannii* is called "pandrug resistant" (PDR), if it is XDR and resistant to polymyxins and tigecycline. Carbapenem resistant *A. baumannii*, which is resistant to carbapenems was ranked as the number one priority for antibiotic research and development in 2018 by the WHO. Carbapenem was chosen as an indicator because carbapenem resistance is often linked to a broad range of other antibiotic resistance (Tacconelli et al. 2018). Northern and Eastern Europe, as well as the Middle East countries of the Arab League (Iraq, Palestine, Lebanon, Jordan, and Syria), have seen an expansion in carbapenem-resistant *A. baumannii* strains. (Moghnieh et al. 2018).

2.5.1 Beta-Lactamases

Antibiotic resistance in *A. baumannii* is mostly based on the inactivation of β-lactams by β-lactamases. β-lactamases are classified into four molecular groups depending on their sequence homology. These are A, B, C, and D (Jeon et al. 2015). Natural ability to assimilate exogenous DNA and high frequency of foreign DNA in *A. baumannii's* genome suggest frequent horizontal gene transfer in this organism (Touchon et al. 2014).

The hydrolysis of clavulanate inhibits the activity of class A β-lactamases. Carbapenems are less effective than penicillins and cephalosporins, except for a few KPC type enzyme. (Jeon et al. 2015). Many β-lactamases of group A, including TEM, SHV, GES, CTX-M, SCO, PER, VEB, KPC and CARB, have been discovered in *A. baumannii* (Moubareck et al. 2009). Class B β-lactamases are metallo-β-lactamases (MBLs) that need zinc or some other heavy metal for catalysis (Jeon et al. 2015). MBLs are able to hydrolyze nearly all β-lactam antibiotics, even carbapenems, because of their wide substrate spectrum. Class C β-lactamases represent a challenge because they can confer resistance to cephamycins, which are commonly used in antibiotic therapy. The AmpC cephalosporinase is fender mental in *A. baumannii* (Gordon and Wareham

2010). Oxacillinases, or Class D β-lactamases, hydrolyze isoxazolylpenicillin oxacillin significantly more quickly than benzylpenicillin (Jeon et al. 2015). More than 400 OXA-type enzymes have been discovered, and many of these have carbapenemase activity as.

2.5.2 Non Enzymatic Mechanism (Efflux Pumps) (EP)

In the Gram negative bacteria, the majority of efflux pumps are composed of three protein components (Nikaido 1996). *A. baumannii's* resistance to antibiotics, including aminoglycosides, qinolones, β-lactamses, carbapenems, chloramphenicol, and macrolides, can be attributed to the presence of efflux pumps in the bacterium, which shield the cells from the detrimental effects of organic compounds (Chopra and Roberts 2001). Efflux pumps of bacteria have a critical part in their pathogenicity. As there are five type of EF, ATP binding cassette (ABC) family,the multidrug and toxic compounds extrusion (MATE), resistance nodulation division (RND), the major facilitator superfamily (MFS) and the small multidrug resistance transporters(SMR). As a result, *A. baumannii* is highly resistant to antimicrobial drugs. For drug proton anti porters, there are a variety of efflux pumps available (Vila, Martí, and Sánchez-Céspedes 2007).

2.5.3 Alteration of Target Sites

Antibiotic resistance in *A. baumannii* can be generated by changes in the antibiotic targeted cells for antibiotics. Only the expression of PBPs changed to have a low affinity for imipenem can lead to imipenem resistance in the absence of any other known resistance mechanisms (Gehrlein et al. 1991). In outbreaks unrelated *A. baumannii* strains, quinolone resistance is linked to changes in GyrA (one component of DNA gyrase) and ParC (one component of topoisomerase IV) (Vila et al. 1995). *A. baumannii* isolates that are resistant to aminoglycosides also include the 16S rRNA methylase ArmA, which always co - exist with carbapenemases like OXA-23 (Hasani et al. 2016). *A. baumannii's* resistance to numerous clinically important antibiotics, such as colistin, can be increased by modifying or removing LPS, as several investigations demonstrates.

2.5.4 Aminoglycoside-Modifying Enzymes

Aminoglycoside altering enzymes change the amino or hydroxyl group as the most common route of resistance to aminoglycosides. *Acinetobacter spp*. has four forms of aminoglycoside modification enzymes, including adenylases, acetylases, methyltransferases, and phosphotransferases. Another pathways of aminoglycosides resistance include decreased drug entrance and alterations in the target ribosomal proteins (Shrestha et al. 2016).

2.5.5 Permeability Defects

Antibiotic resistance can be affected by changes in membrane permeability. In the virulence of *A. baumannii*, porins, for example, generate pathways molecules to be transported through the outer membrane. Porins play an important function in the mechanism of resistance as they influence membrane permeability for example including CarO, and Omp47 (Quale et al. 2003)), is connected with carbapenem resistance in *A. baumannii*. Antibiotic resistance in *A. baumannii* is influenced not only by the outer membrane proteins, but also by components of the membrane, such as LPS and peptidoglycans. Colistin resistance in *A. baumannii* is increased when LPS is lost or modified (Moffatt et al. 2010).

2.6. Colistin

Bacillus spp. produces polymyxins, a lipopeptide antibiotic, while *Bacillus colistinus* produces Polymyxin E (colistin), which was discovered more than 60 years ago and is highly effective against the majority of Gram-negative bacteria (Liu et al. 2014). In clinical practise, only polymyxin B and polymyxin E (colistin) differ by a single amino acid among the five polymyxins (A–E). *A. baumannii* MDR infections are commonly treated with a combination of colistin and other drugs (Cai et al. 2012a). In addition to oral and topical colistin sulphate, the sodium salt of the negatively charged derivative of colistin recognised as colistin CMS or colistimethate sodium is the type of colistin prescribed in the parenteral route (intravenous, intramuscular, intrathecal preparation) and through inhalation, which is an inactive prodrug used for parenteral and nebulization formulations. When taken orally, colistin sulphate is poorly absorbed in the digestive tract and has only a limited ability to kill bacteria in the immediate area. Colistin's chemical reactions, antibacterial activity, mechanism of

action, resistance, pharmacokinetics, pharmacodynamics, and recent treatment application are all being studied and documented. For the treatment of multi-resistant Gram-negative pathogens, colistin is predicted to play a crucial and effective role in the future as an alternative to the antibiotics that have been available so far (Li et al. 2006).

2.6.1. Mechanism action of Colistin

Colistin's primary method of action against Gram-negative bacteria is to target the outer membrane's lipopolysaccharide (LPS). There are lipid bilayers in Gram-negative bacteria cell walls as well as polysaccharides with negative charges, which are stabilised by recombinant cations such as calcium (Ca++) and magnesium (Mg++). There's a layer of peptididoglycan (PG) in between the two membranes. There are five peptides and five amines with polycationic ends in colisin, which can displace the calcium and magnesium ions in the LPS membrane of bacteria and contend their position. It is possible that the binding of colistin peptide to LPS will cause the LPS structure to be destabilized, leading to a decrease in membrane integrity, an increase in membrane penetrability, and ultimately cell death (Falagas, Kasiakou, and Saravolatz 2005).

2.6.2. Antimicrobial Activity of Colistin

There are five colistin's antibacterial mechanisms, (i) colistin's direct antibacterial activity is mediated by electrostatic interactions between its cationic diaminobutyric acid (Dab) residues and the anionic phosphate on the lipid A of LPS in the outer membrane, which disrupts both the microorganisms outer and inner membranes and results in cell lysis (Falagas et al. 2005). (ii) Colistin's anti-endotoxin action, as fatty acid composition in Gram-negative bacteria, a fraction of LPS serves as an endotoxin. By neutralizing and attaching to LPS molecules which inhabiting the endotoxin action, and this will prevents the production of cytokines such as TNF-alpha (TNF-) and interleukin 8 (IL-8), which cause shock, from occurring (Martis, Leroy, and Blanc 2014). (iii) Following passage through the cell layer, colistin interacts with anionic phospholipid vesicles to cause their fusion with the outer surface, leading to phospholipid degradation and necrosis of the organism (Kaye et al. 2016). (iv) Colistin's hydroxyl radical death model. Colistin's Fenton reaction produces reactive

oxygen species (ROS) that destroy DNA, lipids, and proteins, causing cell death; colistin's hydroxyl radical death pathway (Rhouma et al. 2016). (v) Inhibition of respiratory enzymes: colistin's antimicrobial activity is due to its ability to suppress the important respiratory enzymes (El-Sayed Ahmed et al. 2020).

2.6.3. Resistance Mechanism of Colistin

The bacteria's chromosomal DNA encodes the resistance mechanisms of colistin. There are two main methods that have been described. First method, which is caused by mutations in lipid A coding genes, (*lpxA, lpxC,* and *lpxD*) which results in the loss of LPS, an outer component of Gram-negative organisms and a primary target for colistin. (Bojkovic et al. 2015). Second, the pmrAB two-component system, which is a responder regulator and sensors kinase, are involved. Genes essential in lipid A production are regulated in response to changes in pH, Mg^{2+}, and Fe^{3+} concentrations in the environment. Changes in the expression of *pmrA* and *pmrB* are caused by point mutations, and the outer membrane is remodelled as a result (Choi et al. 2017) .

mcr-1, an Escherichia coli plasmid-mediated colistin resistance gene, was recently discovered (McGann et al. 2016). Despite the fact that the *mcr-1* gene is still yet to be discovered in *A. baumannii,* it is predicted that the emergence of transmissible colistin resistance mechanisms will eventually lead to the development from MDR *Acinetobacter spp*. to PDR (Shaheer Ahmed and Alp 2016).

2.6.4. Clinical Usage

To combat MDR Gram-negative bacterial infections when no other treatment choices are available, doctors are increasingly turning to the 60-year-old drug colistin. When it came to Gram-negative bacteria, colistin was a pioneering antibiotic (Nation and Li 2009). Colistin, also known as polymyxin E, was discovered in 1947 by Koyama and has been used since 1959 (Li et al. 2005). Colistin is eliminated by the kidneys via glomerular filtration. Colistin is available in the form of colistimethate sodium, which is delivered intravenously, intramuscularly, and intrathecally and through inhalation. A dose of 2.5 – 5 mg/kg BW/day, distributed into 2-4 doses of Colistin (CMS), is advised for intravenous administration in the United States (1 mg CMS = 12,500 IU). People with normal renal function can take this dose. 4-6 mg/kg BW/day repeated into three doses is the recommended intravenous dose of colistin for adults and children

weighing less than 60 kg in England. A dose of 80-160 mg per eight hours is indicated for those with a BW of more than 60 kg (Falagas et al. 2005).

For bacterial infections or sepsis, colistin has been found to have a strong clinical response, making it a different therapy option. As another treatment for ventilator-associated pneumonia produced by multi-drug resistance organisms such as *P. aeruginosa* or *A. baumannii*, CMS inhalation delivery of colistin can be utilized in the ICU for patients with hospital-acquired-pneumonia. Among the clinical uses of colistin are UTI associated by *A. baumannii*, which can be treated by intravenous CMS of colistin. On the second day of irrigation treatment with 3.5 mg/kgBW of colistin diluted in 500 cc of NaCl, the symptoms of UTI decreased. Injectable CMS bioavailability into cerebrospinal fluid is poor in instances of meningitis caused by multi-drug resistance organisms (such as MDR *A. baumannii*), also use in treatment of osteomyelitis caused by *A. baumannii* MDR. Tablets or syrup containing the antibacterial agent colistin (collistin sulphate) could be used for gastrointestinal infections caused by Gram-negative MDR bacteria. Because of its low intestinal captivation, oral administration of colistin has a good local effect on the gastrointestinal tract and is mostly used to treat diarrhea in children (Yahav et al. 2012). Toxicities must be taken into account when administering oral antibiotics to treat an intestinal infection. Abscess or infection of the skin and soft tissues topical preparations containing colistin sulphate can be used. In addition, there is an ointment and eardrops consist of colistin sulphate are used to treat infections of the eye and ear. Endophtalmitis patients have yet to be treated with colistin due to a lack of data (Michalopoulos and Falagas 2008).

2.6.5. Side Effect of Colistin

Nephrotoxicity and neurotoxicity are two of the most well-known side effects of colistin, however both are reversible and quickly decrease when the treatment has ended. The elderly, those with a history of renal insufficiency, and those undergoing therapy with other nephrotoxic drugs are all at risk for nephrotoxicity (Sorlí et al. 2013). Hematuria, proteinuria, oliguria, acute tubular necrosis and decreased creatinine clearance and elevated urea and creatine serum levels are all clinical indications of the nephrotoxic impact. In addition to monitoring renal function must be considered in the treatment with colistin, method of delivery, and avoiding combination antibiotic

therapy that may produce nephrotoxic side effects (Karvanen 2013). About 11% to 24% of patients receiving colistin therapy developed nephrotoxicity.

It is possible that neurotoxicity can cause respiratory failure or apnea if it causes dizziness, fatigue, facial and peripheral paraesthesia, vertigo, loss of vision or ataxia. Inhalation can produce minor bronchoconstriction in patients who are particularly sensitive. The use of high doses of colistin, especially intraventricular and intrathecal, must be constantly controlled because it can lead to seizures (Falagas *et al*; 2005). Hypersensitivity reaction, rash, urticarial, pruritus, and muscle weakness in all parts of the body, as well as a moderate digestive condition, are all possible side effects of colistin therapy. Studies have shown that patients with cystic fibrosis are more susceptible to experience neurotoxic consequences. The adverse effects of colistin therapy were reversible and reduced when the medication was withdrawn, with a neurotoxicity rate of about 7% and a primary symptom of Paranesthesia. *(Tamma et al.* ; 2013)

CHAPTER III

3. MATERIAL AND METHODS

3.1. Tools and Equipment

- Petri plates
- Microplate with sterile cover
- Automatic pipette
- Loop
- Weigh Gram Digital Scale
- Autoclave
- Incubator
- Spectrophotometer (McFarland stander)

3.2. Kits and Chemicals

BD, BBL ™ Blood Agar Base: we suspended 40g of the powder in 1 L of purified water then autoclaved for 15 min with temp 121°C. At 50°C, then we added 5% sterile, defibrinated blood and after that needed to cool the base to 45 °C. Then we poured the solution in petri plates and wait to cool and use for culture.

Mueller Hinton Broth2 (90922), Cation-Adjusted (M-H 2 Broth; Mueller Hinton II Broth) (CAMHB) (Merck-Sigma-Aldrich): MHB preparation based on commercial instructions to suspend 22 g in 1 liter distilled water, heated if necessary to dissolve the medium completely. Dispense and sterilize by autoclaving at 10-15 Ibs pressure (115-121°C) for 10 min. with do not overheating and mix well before pouring. The medium is supplement with appropriate salts to provide 20-25 mg/L calcium and 10-12.5 mg/L Magnesium.

Preperation of Colistin Sulphate: Colistin sulphate (C2700000) (25 mg) (Merck -Sigma-Aldrich). To prepare colistin solution for this, 25 mg colistin were dissolved in 4.882 ml sterile H_2O. Then stored at -20 °C. Then 1 ml colistin sulphate added to 9 ml sterile water the concentration will be 512 mg/L (1ml contain 5.12 mg)

3.3. Collection of Bacterial Isolates

The total of 50 *A. baumannii* complex strains obtained from laboratories of Near East University Hospital North Cyprus, which collected from different clinical departments, between January 2012 and August 2021 were included in this investigation. The isolated strains were stored at a temperature of -20°C in bacteria storage tubes.

3.4. Culture

The isolation of *A. baumannii* which were stored at a temperature of -20°C in bacteria storage tubes we take them and put in the room temperature until melting and then cultured on the blood agar and incubated for 24h at 35°C (Figure 3).

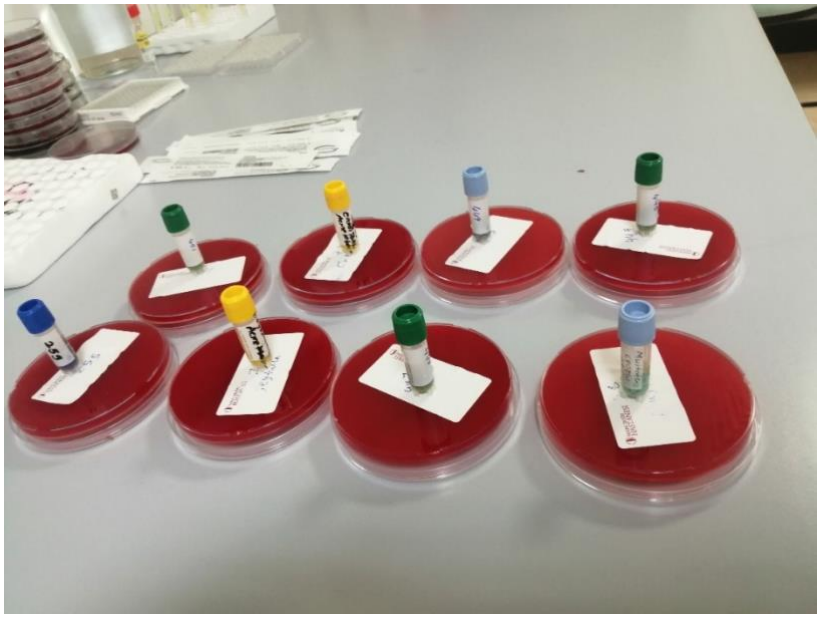


Figure 3. *Preparing A. baumannii To Be Cultured On Blood Agar At NEU 'S Lab*

3.5. Identification of Bacteria

The identification of Acinetobacter infection is made by the growth of Acinetobacter, which isolated from patients specimen (e.g. sputum, blood, cerebrospinal fluid, etc.*). Acinetobacter spp*. appear Gram-negative and coccobacilli in shape. On blood agar non-hemolytic, opaque, circular, and gray color colonies will be observed (Figure 4).

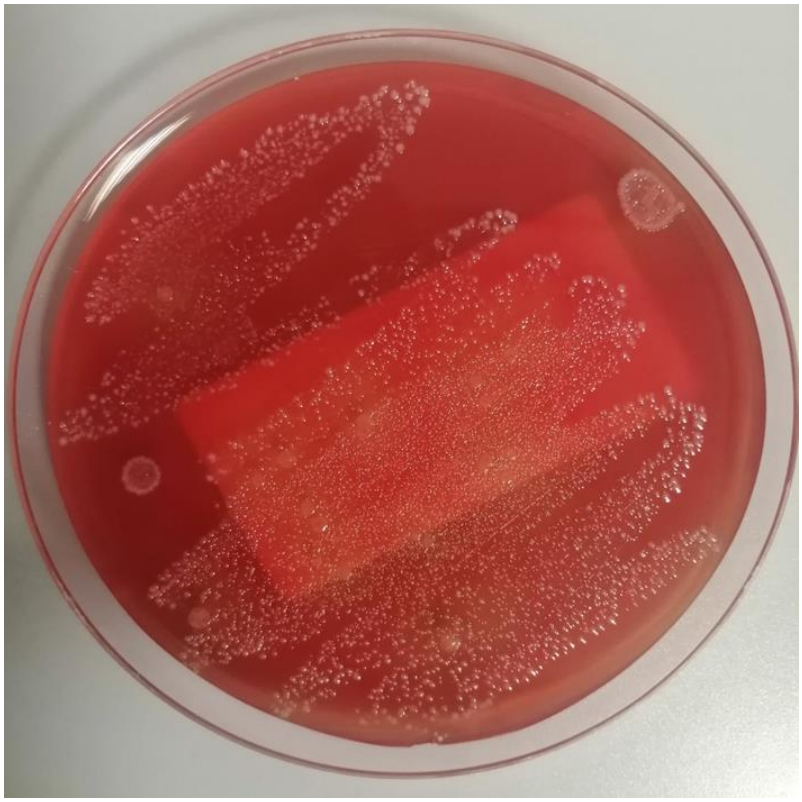


Figure 4. *A. baumannii Strains On Blood Agar After Culturing 24 H At NEU's Lab*

3.6. Antimicrobial Susceptibility Test by Broth Microdilution Method

To determine the Microdilution concentration of colistin, we used dilution methods that attempt to find the lowest concentration that inhibits noticeable bacterial growth after 16 to 24 hours of incubation at 35 ± 2°C.

1. The dilution ranges of colistin to be studied with using microplate are determined (0.125 -128 mg/L). This dilution antibiotic stock solution is prepared according to the intervals.

- First, the stock solution is prepared. For this, 51.2 mg colistin was weighed. Dissolve in 10 ml steriled H_2O. 1 ml aliquots are stored at -20 °C.
- 4.882 ml + 25 mg , then 1 ml colistin + 9 ml water as a result the concentration will be 512 mg/L (1ml contain 5.12 mg)
- The concentration of colistin in the first well is 128 mg/L 4 times dense (512 mg/L)

2. Each well 100 µL is added from CAMHB

3. Then, the first microplates containing 100 µl of CAMHB in each well, add 100 µl of the prepared antibiotic solution to the wells in the column. With the help of a micropipette, 100 µl is taken from the first wells transferred to the second wells, and pipetted. By transferring into third wells from second wells double-layer dilutions of the antibiotic are obtained, is done. 100 µl from the eleventh well is expelled.

4. Since the twelfth wells will be a microorganism growth control wells, Antibiotics are not placed in these wells.

5. Then the *Acinetobacter spp.* suspension was prepared in accordance to the manufacturer's standard density (0,45-0,55 McFarland) by using a spectrophotometric device.

6. 100 µl of *Acinetobacter spp* were added to all wells.In each well became 100 µl of CAMHB + 100 µl antibiotic solution + 100 µl Acinetobacter susp

7. Necessarily quality control strains (colistin-susceptible *E. coli* ATCC 25922 or *P. aeruginosa* ATCC 27853 and resistant *E. coli* NCTC 13846 included in the study should be done.

8. Microplates were incubated at 35±2 °C for 24 hours.

9. At the end of the appropriate incubation period, microorganism growth is not observed in the well has (the lowest that the antibiotic concentration inhibits growth) in this case antibiotic concentration is determined as the MIC value.

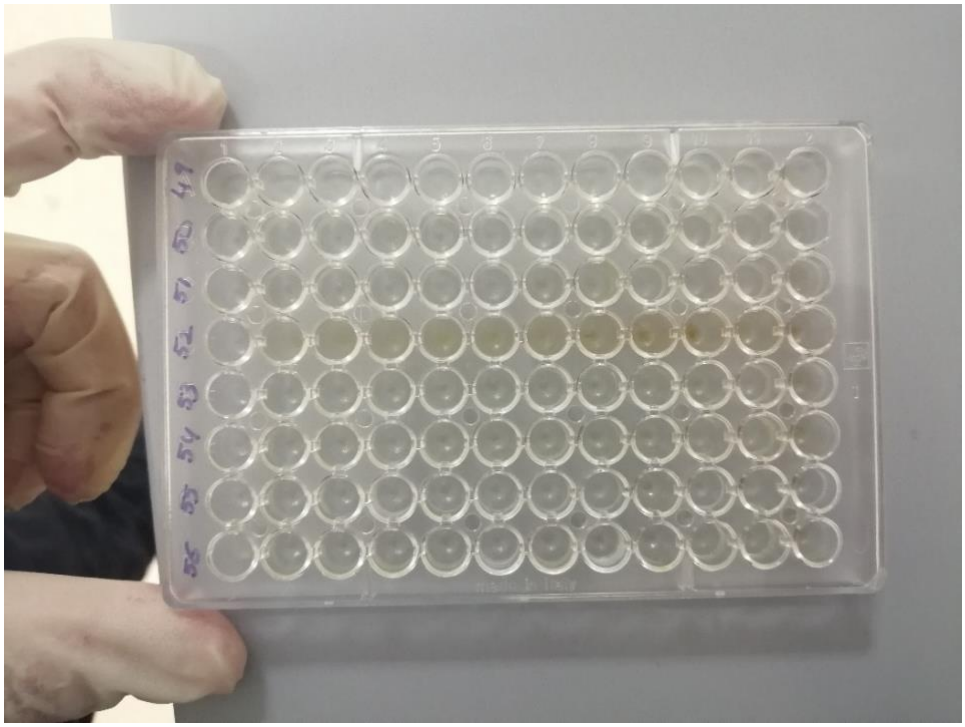


Figure 5. *Microplate With One Sample Resistant To Colistin.*

3.7. Statistical Analysis

All results and analysis of data done by using IBM SPSS v28.1.1 and MS Excel programs. We tried to determine the percentage of the resistance samples, percentage of male and female to compare it with previous studies, determine the mean according to our sample, and we tried to show the distribution of specimens in different department of NEU's hospital, then to compare our result with previous studies around the world.

CHAPTER IV

4. RESULTS

In our research out of 50 samples we found that 74 % (n: 37) of the specimens male, while 26% (n: 13) female. There was a wide spectrum of ages in attendance (between 24 -91). And the mean was 65 in our study according to ages of our samples.

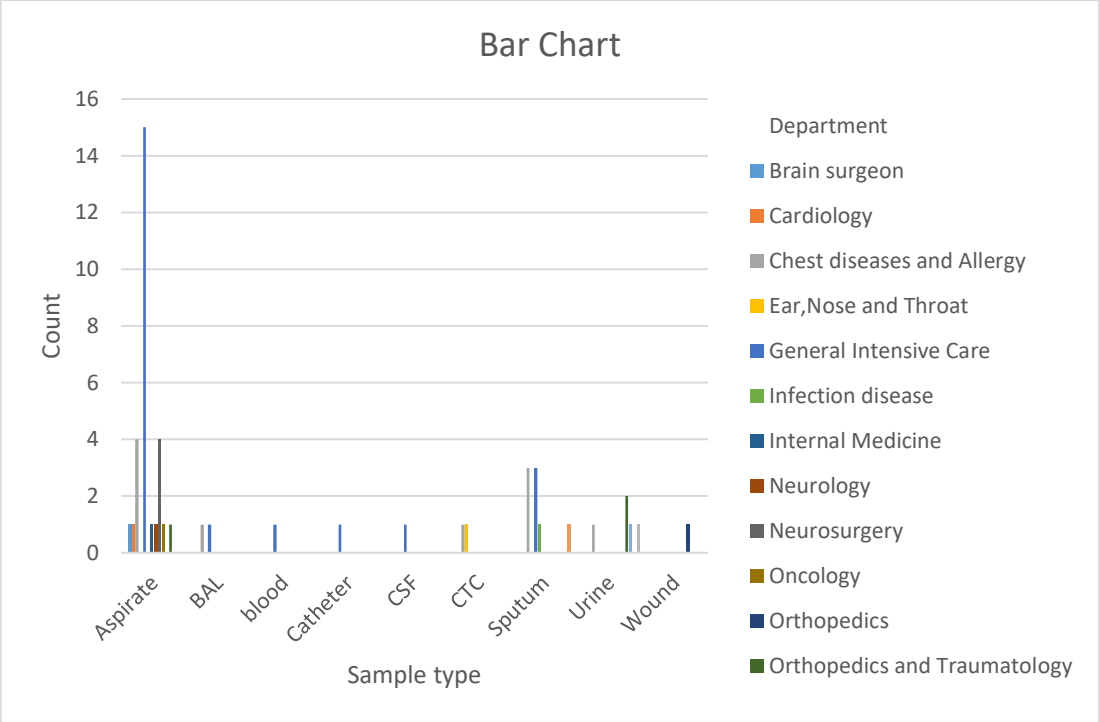
Fifty *Acinetobacter spp*. specimens were obtained. 58% (n=29) aspirate specimens, 16% (n=8) sputum, and 4% (n=2) from Broncho alveolar lavage (Table 1).

Table 1. *The Distribution Of Specimens*

	Number	%
Aspirate	29	58.0
BAL	2	4.0
Blood	1	2.0
Catheter	1	2.0
CSF	1	2.0
CTC	2	4.0
Sputum	8	16.0
Urine	5	10.0
Wound	1	2.0
Total Number	50	100.00

Figure 6 showed the distribution of specimens' types taken from all departments and reveal that 15 aspirate (33 %), were isolated from the general ICU.

Figure 6. *The Distribution Of Specimens From Different Departments*



According to the EUCAST guidelines for *A. baumannii* isolate sensitivity testing of colistin, a MIC ≤2 µg/mL is considered sensitive, while a MIC of >2 µg/mL is considered resistant, according to what mentioned above we found 1 strain (2%) was resistant and 49 strains were (98%) were sensitive (Table 2).

Table 2. *The Result Of Susceptibility Testing Of Colistin Determine By Broth Microdilution Method*.

	Number	%
Resistant	1	2.0
Sensitive	49	98.0
Total	50	100.0

Table 3. *The Total Data Of Patients Included In This Study*

	Gender	Age	Date	Department	State	Sample_NO	Sample type	Colistin (MIC)
1	Male	64	2020	General Intensive Care	Inpatient	1867829	Aspirate	resistant
2	Male	78	2020	Brain surgeon	Inpatient	1770678	Aspirate	sensitive
3	Male	61	2020	Cardiology	Inpatient	1793699	Aspirate	sensitive
4	Male	60	2012	Chest diseases and Allergy	Inpatient	1996	BAL	sensitive
5	Male	68	2012	Chest diseases and Allergy	Inpatient	16963	Aspirate	sensitive
6	Male	82	2014	Chest diseases and Allergy	Inpatient	90677	Sputum	sensitive
7	Male	76	2014	Chest diseases and Allergy	Inpatient	88621	Aspirate	sensitive
8	Female	61	2014	Chest diseases and Allergy	Inpatient	462779	Sputum	sensitive
9	Male	91	2015	Chest diseases and Allergy	Inpatient	85024	Aspirate	sensitive
10	Male	75	2015	Chest diseases and Allergy	Inpatient	657233	Urine	sensitive
11	Male	55	2015	Chest diseases and Allergy	Inpatient	658273	CTC	sensitive
12	Male	55	2015	Chest diseases and Allergy	Inpatient	659878	Aspirate	sensitive
13	Female	24	2020	Chest diseases and Allergy	Inpatient	1868458	Sputum	sensitive
14	Male	62	2012	Ear,Nose and Throat	Inpatient	14701	CTC	sensitive
15	Male	73	2019	General Intensive Care	Inpatient	1749092	Aspirate	sensitive
16	Male	69	2020	General Intensive Care	Inpatient	1762350	BAL	sensitive
17	Male	84	2020	General Intensive Care	Inpatient	1789247	Aspirate	sensitive
18	Male	87	2020	General Intensive Care	Inpatient	1825936	Aspirate	sensitive
19	Female	86	2020	General Intensive Care	Inpatient	1836038	Catheter	sensitive
20	Female	64	2020	General Intensive Care	Inpatient	1836957	Aspirate	sensitive
21	Male	82	2020	General Intensive Care	Inpatient	1837038	CSF	sensitive
22	Male	87	2020	General Intensive Care	Inpatient	1836731	Sputum	sensitive
23	Male	50	2020	General Intensive Care	Inpatient	1841393	Sputum	sensitive
24	Female	83	2020	General Intensive Care	Inpatient	1848744	Aspirate	sensitive
25	Female	83	2020	General Intensive Care	Inpatient	1848745	blood	sensitive
26	Male	65	2020	General Intensive Care	Inpatient	1851727	Aspirate	sensitive
27	Male	63	2020	General Intensive Care	Inpatient	1852091	Aspirate	sensitive
28	Male	43	2020	General Intensive Care	Inpatient	1852460	Aspirate	sensitive
29	Male	73	2020	General Intensive Care	Inpatient	1856528	Sputum	sensitive
30	Male	43	2020	General Intensive Care	Inpatient	1860498	Aspirate	sensitive
31	Male	64	2020	General Intensive Care	Inpatient	1867203	Aspirate	sensitive
32	Male	73	2020	General Intensive Care	Inpatient	1871505	Aspirate	sensitive
33	Male	73	2020	General Intensive Care	Inpatient	1857534	Aspirate	sensitive
34	Female	79	2020	General Intensive Care	Inpatient	1877970	Aspirate	sensitive
35	Male	73	2020	General Intensive Care	Inpatient	1878007	Aspirate	sensitive
36	Female	26	2014	Infection disease	Inpatient	461837	Sputum	sensitive
37	Female	57	2015	Internal Medicine	Inpatient	652962	Aspirate	sensitive
38	Male	62	2012	Neurology	Inpatient	54575	Aspirate	sensitive
39	Male	30	2012	Neurosurgery	Inpatient	46221	Aspirate	sensitive
40	Female	81	2012	Neurosurgery	Inpatient	46905	Aspirate	sensitive
41	Female	81	2012	Neurosurgery	Inpatient	46905	Aspirate	sensitive
42	Male	38	2012	Neurosurgery	Inpatient	49923	Aspirate	sensitive
43	Female	75	2018	Oncology	Inpatient	9849	Aspirate	sensitive
44	Male	26	2012	Orthopedics	Inpatient	54600	Wound	sensitive
45	Male	66	2012	Orthopedics and Traumatology	Inpatient	16498	Aspirate	sensitive
46	Male	66	2012	Orthopedics and Traumatology	Inpatient	16498	Urine	sensitive
47	Female	73	2020	Orthopedics and Traumatology	Inpatient	1835810	Urine	sensitive
48	Male	50	2012	Physiotheraphy	Inpatient	1569	Urine	sensitive
49	Male	73	2013	Polyclinic of Cardiology	Inpatient	73119	Sputum	sensitive
50	Male	71	2015	Urology	Inpatient	652646	Urine	sensitive

CHAPTER V

4. DISCUSSION

The Infectious Diseases Society of America (IDSA) has designated *A. baumannii* as one of the most harmful nosocomial microorganisms. Since MDR *A. baumanni* isolates have been obtained all over the world, colistin has become a therapy option for infections caused by this bacteria (Cai et al. 2012).

Fifty samples of *A. baumannii* specimens were obtained from Near East University Hospital Microbiology Laboratory. 58 % (n=29) of the isolates from aspirate specimens, 16% (n=8) from sputum, and 4% (n=2) from broncho alveolar lavage, that mean 78% (n=39) of *A. baumannii* isolates were found in respiratory collections of all. 44% of the samples came from the general ICU.

According to Çağlan *et al.*, they studied 200 *A. baumannii* isolates in Turkey,which 48% came from respiratory specimens (bronchoalveolar lavage, aspirate, and sputum) the overall resistance to colistin was found to be 28%. Also, they found 4.2% colistin resistance by E-test and 25.8% by broth microdilution method (Çağlan et al. 2019)**.**

Another study, 177 bacterial strains were isolated from a variety of clinical specimens between March 2010 and December 2012 (77 strains *A.baumannii*/60 strains *P.aeruginosa*) in Turkey. They used Vitek 2 Compact (bioMérieux, France) and BD Phoenix 100 (Becton Dickinson, USA) systems to identify and assess antibiotic susceptibility of the isolates. Colistin resistance rate was 4 % (Mengeloğlu et al. 2014). Hundred stains of *A. baumannii* from blood culture isolates were tested according to. The CLSI broth microdilution method was used to assess the susceptibilities of the isolates to antimicrobial agents. Colistin, susceptibilities were tested using E-test and they reported the colistin resistance was 2 % (Ergin, Hascelik, and Eser 2013). Broth microdilution method of colistin for Gram-negative bacteria from the ICUs of Taiwan's seven major teaching hospitals in 2016.There were found n=14 (10.1%) resistant *Acinetobacter baumannii* complex isolates in Taiwan out of 138 (Lai et al. 2019).

An antimicrobial surveillance programme, SENTRY between 2001 and 2011 Antimicrobial Surveillance, found that *A. baumannii's* colistin resistance remained at

a limited level (0.9%- 3.3%) (Yau et al. 2009). There is a 6.1% prevalence of *A. baumanni* colistin resistance in European countries according to the SENTRY survey, from 2013 -2016. This percentage increase to 10.4%. Turkey is one of the most resolute countries in the world (Çağlan et al. 2020). Over the course of 18 months, a total of 96 *A. baumannii* clinical isolates were obtained from the Düzce University Hospital in Turkey. Sixty-one patients (63.5 %) were in the ICU. Most of the isolates were found in respiratory specimens (tracheal aspirates (54.2%), sputum (12.5%), bronchoalveolar lavage (5.2%)). They used Phoenix Automated System and PCR. Colistin resistance was not found in any isolate (0%) (Say Coskun et al. 2019).In contrast, in a Korean study, when the broth microdilution test was used, the colistin resistance was reported as 30.6% (Ko et al. 2007). Also 31 of 65 *A. baumannii* isolates isolated from patients with ventilator-associated pneumonia enrolled in the MagicBullet study in Europe were resistant to colistin. the results were: Greece had the largest percentage of colistin-resistant isolates (56.8%), followed by Italy (42.9%) and Spain (42.9%). (28.6 %).(Nowak et al. 2017). In our study, 1 strain (2%) was resistant and 49 strains were (98%) were sensitive.

CHAPTER VI

5. CONCLUSION

Colistin has garnered a lot of attention recently as maybe a last alternative for treating MDR *A. baumannii*. Although there have been many reports of resistance to colistin all throughout the world. Colistin is still good choice until now as antibiotic to treatment *Acinetobacter* needs more studies to do and find ways to reduce its side effects.

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APPENDICES

Appendix A

YAKIN DOĞU ÜNİVERSİTESİ
BİLİMSEL ARAŞTIRMALAR ETİK KURULU

ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi : 30.09.2021
Toplantı No : 2021/95
Proje No :1402

Yakın Doğu Üniversitesi SHYMO öğretim üyelerinden Doç. Dr. Meryem Güvenir'in sorumlu araştırmacısı olduğu, YDU/2021/95-1402 proje numaralı ve **"Çeşitli Klinik Örneklerden İzole Edilen *Acinetobacterbaumannii* Suşlarında Kolistin Direncinin Araştırılması"** başlıklı proje önerisi kurulumuzca değerlendirilmiş olup, etik olarak uygun bulunmuştur.

Prof. Dr. Şanda Çalı
Yakın Doğu Üniversitesi
Bilimsel Araştırmalar Etik Kurulu Başkanı

CURRICULUM VITAE

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National	SYRAIN	**Tel**	00905347242925
E-mail	An.br237@gmail.co		

Educational Level

	Name of the Institution where he/she	**Graduati**
Postgraduate/S		
Masters		
Undergraduate	Damascus university	2009
High school		

Job Experience

	Duty	**Institution**	**Duration**
	Program Medical Manager	UOSSM medical	2018- 2022
	Regional Manager	UOSSM medical	2014-2018
	Pharmacist	Nesrin pharmacy	2013-2014
	Pharmacist /Part time	Almarwan pharmacy	2010-2011
	Student at ministry of health	Directorate of medicine	2009-2012

Foreign	**Reading**	**Speaking**	**Writing**
English	Very good	Very good	Very good
Turkish	good	good	good
Arabic/mother	Excellent	Excellent	Excellent

Computer Knowledge

Program	**Use proficiency**
MS Programs	Very good