TURKISH REPUBLIC OF NORTHERN CYPRUS

NEAR EAST UNIVERSITY

HEALTH SCIENCES INSTITUTE

INVESTIGATION OF ANTIBIOTIC RESISTANCE, ESBL, AND BIOFILM PROPERTIES OF *ACINETOBACTER* SPECIES STRAINS ISOLATED FROM VARIOUS CLINICAL SAMPLES

MOHAMMAD HEIDER SALEH MALKAWI

MASTER OF SCIENCE THESIS

MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY PROGRAM

2020 – NICOSIA

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MENTOR

ASSOC. PROF. DR. MERYEM GÜVENIR

CO-ADVISOR

DR. EMRAH GÜLER

2020 – NICOSIA

The Directorate of Health Sciences Institute,

This study has been accepted by the Thesis Committee in Medical and Clinical Microbiology Program as a Master of Science Thesis.

Thesis committee:

Chairman:

Prof. Dr. Kaya Süer
Near East University, Faculty of Medicine,
Department of Clinical Microbiology and Infectious Diseases

Members:

Assoc. Prof. Dr. Meryem GÜVENIR
Near East University, Vocational School of Health Services,
Department of Medical and Clinical Microbiology

Assist. Prof. Dr. Özel YÜRÜKER
Girne University, Faculty of Medicine
Department of Clinical Microbiology and Infectious Diseases

Dr. Emrah GÜLER
Near East University, Faculty of Health Sciences

Approval:

According to the relevant articles of the Near East University Postgraduate study-Education and Examination Regulations, this thesis has been approved by the abovementioned members of the thesis committee and the decision of the Board of Directors of the institute.

Professor Dr. K. Hüsnü Can BAŞER, PhD
Director of Graduate School of Health Sciences

STATEMENT (DECLARATION)

The data presented in this thesis was obtained in an experiment carried out in the microbiology laboratory/Near East University Hospital. I played a major role in the preparation and execution of the experiment, and the data analysis and interpretation are entirely my own work.

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MOHAMMAD HEIDER MALKAWI

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Date: 07-09-2020

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LIST OF ABBREVIATIONS

%: Percent sign
°C: Celsius
A. baumannii: *Acinetobacter baumannii*
ABC: ATP-Binding Cassette
Acb: *Acinetobacter calcoaceticus*, *Acinetobacter baumannii*
AHL: Acyl-homoserine Lactones
AIF: Apoptosis-inducing factor
AK: Amikacin
AMC: Amoxicillin- Clavulanic Acid
AMEs: Aminoglycoside Modifying Enzymes
Amp: Ambler membrane protein
ASTs: Antibiotic susceptibility tests
ATCC: American type culture collection
ATM: Aztreonam
ATP: Adenosine triphosphate
BAL: Bronchoalveolar Lavage
BfmRS: Biofilm Regulating System
BHI: Brain Heart Infusion Broth
BSIs: Bloodstream infections
C: Cytosine
CAZ: Ceftazidime
CDC: Centers for Disease Control and Prevention
CFP: Cefepime
CIP: Ciprofloxacin
COL: Colistin
CRA: Congo Red Agar
CRAB: Carbapenem-Resistant *Acinetobacter baumannii*

CRISPR: Clustered Frequently Interspaced Short Palindromic Repeats
CRO: Ceftriaxone
CSF: Cerebrospinal Fluid
CTX: Cefotaxime
CTX-M: Cefotaximase
CTZ: Ceftazidime
DDST: Double-Disc Synergy Test
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
FEP: cefepime
FOX: cefoxitin
G: Gentamicin
g: gram
G: Guanine
H_2S: Hydrogen Sulfide
hr: hour
ICU: Intensive care unit
IMI: Imipenem
IMP: Imipenem metallo-beta-lactamase
iNOS: increases nitric oxide synthase
IV: Intravenous
LOS: Lipooligosaccharide

LPS: Lipopolysaccharide
MBL: Metallo-Beta-Lactamase
mcg /µg: Microgram
MCRA: Modified Congo Red Agar
MDR: Multi-Drug Resistant
MDRAB: Multi-Drug Resistant *Acinetobacter baumannii*
MER: Meropenem
mg/kg: milligram per kilogram
mg: milligram
MHA: Mueller-Hinton Agar
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
OST: Oligosaccharyltransferases
OXA: Oxacillinase
PBPs: penicillin-binding proteins
PCR: Polymerase chain reaction
PDR: Pan-Drug Resistant
PER: Pseudomonas Extended-Resistant
pEtN: phosphoethanolamine
pH: Potential for hydrogen
PNAG: Poly-beta-1-6-N-acetylglucosamine
POD: Cefpodoxime

P-value: Probability value
QC: Quality control
QS: Quorum Sensing
R: Resistant
RNA: Ribonucleic acid
RND: The resistance-nodulation-cell division
RPPs: Ribosomal Protection Proteins
S: Susceptible
SD: Standard Deviation
SHV: Sulfhydryl Variable
SIM: Seoul Imipenemase
SPSS: Statistical Package for the Social Sciences
TCS: two-component system
TEM: Temoneira
TFP/Csu: Type IV pili
TLR2: Toll-like receptor 2
TRNC: The Turkish Republic of Northern Cyprus
Und-P: Undecaprenyl phosphate
units/kg: units per kilogram
UTI: Urinary tract infection
VEB: Vietnamese Extended-spectrum Beta-lactamase
VIM: Verona Integron-encoded Metallo-beta-lactamase
vtx: Vero-toxins
XDR: Extensively-Drug Resistant
µg/mL: Microgram per Milliliter
µm: Micrometer

ABSTRACT

Mohammad Heider Saleh Malkawi. Investigation of Antibiotic Resistance, ESBL, And Biofilm Properties of *Acinetobacter* Species Strains Isolated from Various Clinical Samples. Near East University, Institute of Health Sciences, Medical Microbiology and Clinical Microbiology Program, M.Sc. Thesis, Nicosia, 2020

Aim: This study was conducted to determine the antibiotic resistance, ESBL (Extended Spectrum Beta-Lactamase), and biofilm properties of *Acinetobacter* species that isolated from various clinical samples and to determine the associated connection between these virulence factors.

Materials and Methods: A total of 93 isolated samples were from hospitalized patients which were identified for *Acinetobacter* species and tested for Antibiotic Susceptibility Tests (ASTs) throughout full automated system Phoenix 100. ESBL production was accomplished by Double-Disc Synergy Test (DDST) method. Biofilm formation was performed by Congo Red Agar (CRA) method.

Results: Distribution of specimens were 62,4% (n: 58) isolated from males and 37,6% (n: 35) from females, old adults with 88,2% (n: 82) were the most age category that infected samples collected from, and the majority of isolated specimens were aspirate 48,4% (n: 45). The results of ASTs were 76,3% amikacin resistant, 82,8% ciprofloxacin resistant, 77,4% gentamicin resistant, 81,7% imipenem resistant, 81,7% levofloxacin resistant, 82,8% meropenem resistant, 68,8% trimethoprim/sulfamethoxazole resistant, and 6,5% colistin resistant. In the investigation of ESBL production among *Acinetobacter* species, this study shows 22,6% (n: 21) positive ESBL. Among the investigation of biofilm formation in *Acinetobacter* species, this study shows 93,5% (n: 87) biofilm producers.

Conclusion: The conspicuous prevalence of biofilm-forming and multidrug-resistant of *Acinetobacter* bacteria in our institutions provides a glimpse of potential challenges in our region of the world. Routine monitoring of biofilm formation and beta-lactamases should be taken into consideration in the therapy plan.

Key Words: *Acinetobacter*, Antibiotic susceptibility test, ESBL, Biofilm

Özet

Mohammad Heider Saleh Malkawi. Çeşitli Klinik Örneklerden İzole Edilen Acinetobacter Türlerinin Antibiyotik Direnci, ESBL ve Biyofilm Özelliklerinin Araştırılması. Yakın Doğu Üniversitesi, Sağlık Bilimleri Enstitüsü, Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji Programı, Yüksek Lisans Tezi, Lefkoşa, 2020

Amaç: Bu çalışma, çeşitli klinik örneklerden izole edilen *Acinetobacter* türlerinin antibiyotik direncini, GSBL (Genişlemiş Spektrumlu Beta-Laktamaz) ve biyofilm özelliklerini belirlemek ve bu virülans faktörleri arasındaki ilişkiyi belirlemek amacıyla yapılmıştır.

Gereç ve Yöntemler: Hastanemizde yatan hastalardan izole edilen ve tam otomatize sistem Phoenix 100 ile tanımlanıp, Antibiyotik Duyarlılık Testleri (ADT) test edilen toplam 93 *Acinetobacter* spp. türü çalışmaya alındı. ESBL üretimi Çift Disk Sinerji Testi (ÇDST) yöntemi ile gerçekleştirildi. Biyofilm oluşumu Kongo Red Agar (KRA) yöntemi ile yapıldı.

Bulgular: Örneklerin %62,4 (n: 58)'ü erkeklerden ve %37,6 (n: 35)'sı kadınlardan izole edilmiştir. Örneklerin %88,2 (n: 82) ile çoğunluğu yaşlı yetişkinlerde saptanmış ve *Acinetobacter* türlerinin en sık aspirat numunelerinden % 48,4 (n: 45) izole edildiği tespit edilmiştir. Antibiyotik duyarlılık testlerinde elde edilen direnç oranları: %76,3 amikasin dirençli, %82,8 siprofloksasin dirençli, %77,4 gentamisine dirençli,% 81,7 imipenem dirençli, %81,7 levofloksasine dirençli, %82,8 meropenem dirençli, %68,8 trimetoprim/sulfametoksazole dirençli ve %6,5 colistin dirençli şeklindedir. *Acinetobacter* türleri arasında %22,6 (n: 21)'sının GSBL pozitif olduğu görülmektedir. *Acinetobacter* türlerindeki biyofilm oluşumunun araştırılmasında ise, % 93,5 (n: 87) oranında biyofilm üreten suşa rastlanmıştır.

Sonuç: Biyofilm oluşturan ve çoklu ilaca dirençli *Acinetobacter* bakterilerinin dikkat çeken prevalansı, dünyada potansiyel zorluklara neden olmaktadır. Bu sebeple, tedavi planında biyofilm oluşumu ve beta-laktamazların rutin olarak izlenmesinin gerekli olduğunu düşünmektedyiz.

Anahtar Kelimeler: *Acinetobacter*, Antibiyotik duyarlılık testi, ESBL, Biyofilm

1. INTRODUCTION

Acinetobacter is a genus of bacteria (germs) found in the environment, such as soil and water. *Acinetobacter* species easily thrive on common growth media, both solid and liquid. The *Acinetobacter* genus essentially thrives on solid agars after 18-24 hour in 25°C to 45°C incubation. 37°C is the optimal temperature for this species to cultivate in which human infection obtained in this temperature, but 30°C and below this temperature are optimal for species in the environment. The colony of *Acinetobacter* appears smooth, gray to white, and often appears mucoid with (1,5 mm to 3 mm) diameter which similar to the Enterobacteriaceae family (Antunes et al., 2011; Peleg et al., 2008). For biochemical tests, nearly all *Acinetobacter* species were not able to generate acid from mannitol and sucrose but were able to generate it from glucose, lactose, galactose, mannose, rhamnose, and xylose for biochemical processing. These species were positive for Simmons Citrate and were negative for the development of hydrogen sulfide (H_2S), indole, Voges-Proskauer tests, nitrate, and esculin hydrolysis (Constantiniu et al., 2004). *Acinetobacter* species are able to break down herbicides, alkanes, and few of the pharmaceutical compounds which are chemical substance naturally or not naturally found within an organism these compounds named as xenobiotic (Antunes et al., 2014; Tenover, 2006).

Acinetobacter species may be incorrectly identified as Gram (-) or Gram (+) cocci due to their difficulties in de staining because species of *Acinetobacter* have similar of various features among each, in Gram staining resembles Gram (-) Cocco-bacilli, diploid or variable-length chains forming (Peleg et al., 2008). *Acinetobacter* species have in their component (guanine G + cytosine C) content that changes from thirty-nine to forty-seven percent. Although have classified as aerobic bacteria, not fermentative bacteria, and not motile (Rossau et al., 1991).

The aim of this study, investigate the antibiotic resistance, ESBL, and biofilm properties of *Acinetobacter* species that isolated from various clinical samples and to determine the associated connection between these virulence factors.

2. GENERAL INFORMATION

2.1. Taxonomy and History

Acinetobacter is a genus of bacteria (germs) found in the environment, such as soil and water. Although there are multiple forms of infection, the Centers for Disease Control and Prevention (CDC) notices that the most prevalent cause of infections specifically in humans is *Acinetobacter baumannii* (*A. baumannii*), and it is associated with infections acquired from hospitals that involve ventilator-associated pneumonia, bloodstream, meningitis, and urinary tract infections (UTI) (CDC, 2019; Handal et al., 2017).

The genus *Acinetobacter* has a long history, and the difference between different species has only recently become possible. According to the phylogenetic classification that positions the genus *Acinetobacter* in the Proteobacteria Gammaproteobacteria (Juni, 2005). The Dutch microbiologist Martinus Willem Beijerinck who help to discover the origin of the *Acinetobacter* genus in 1911 when analysis soil samples and isolates the microorganism using enriching in mineral media holding either minimally medium including calcium acetate or quinate and aerobically incubated which he called it calcoaceticus Micrococcus (Beijerinck, 1911). Over the following decades, distinct genera and species have been reported and defined to similar organisms, for example: *Achromobacter anitratus* (Brisou, 1953), *Moraxella lwoffi* (Audureau, 1940), *Bacterium anitratum* (Schaub & Hauber, 1948), *Achromobacter mucosus* (Mannheim and Stenzel, 1962), *Diplococcus mucosus* (Von Lingelsheim, 1908), *Neisseria winogradskyi* (Lemoigne et al., 1952), *Alcaligenes haemolysans* (Henriksen, 1973), and *Herellea vaginicola* (DeBord, 1942). And until the 1970s, many species and generas have been described which have later some of them classified as *Acinetobacter*, and all of these different names were epitomized by Henriksen in 1973 (Henriksen, 1973). Brisou and Prévot suggested the name Akinetos -which in the Greek language means nonmotile- matching the *Acinetobacter* genus in 1954 to distinguish when the microorganisms have (motility) movable and when they are not within the *Achromobacter*. Then in 1968

Baumann conducted a thorough review and confirmed that the spp. discussed earlier related to the established *Acinetobacter* genus based on their morphological properties (Baumann et al., 1968). According to Baumann, the definition and the differentiation of *Acinetobacter* was classified by (Subcommittee on Taxonomy of Moraxella and Allied Bacteria in 1971) from *Achromobacter* genus (Lessel, 1971). Interestingly, in 1969 the first *Acinetobacter* specimen was taken from the general intensive care (ICU) (Stirland et al., 1969). *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii* have been classified into acids generators and nonacids generators of sugars retrospectively which passed on "Approved List of Bacterial Names" at end of 1980s (Skerman et al., 1980). This genus of bacteria was placed in the *Moraxellaceae* family in 1991. Then proposed in *Pseudomonadales* order, *Gammaproteobacteria* class, and genus *Acinetobacter* (Rossau et al., 1991). Since *Acinetobacter* was found in 1911, the bacteria has gone through difficult and various nomenclature (Peleg et al., 2008). This is mainly due to the fact that species of Acinetobacter capable of adjusting to nearly all substances via specific catabolic methods hence, cause difficulties in biochemical examination (La Scola et al., 2006). 12 GS were outlined based on DNA-DNA interaction which had a DNA-DNA interaction of more than 70%. Those genospecies resulted as *A. baumannii*, *A. calcoaceticus*, *A. lwoffii*, *A. junii*, *A. johnsonii*, and *A. haemolyticus* (Bouvet and Grimont, 1986). At the time not all the organisms found were given a name when they were first discovered. A total of thirty one genomic spp. were described in subsequent years; seventeen of the obtained were named. "Genomic Species (GS)" associated with a number which may differ based on the nomenclature pattern were named for the others (Bouvet and Jeanjean, 1989; Carr et al., 2003; Nemec et al., 2001, 2003; Nishimura et al., 1988; Tjernbergt and Ursing, 1989). The number of genomic spp. that identified has risen to thirty sex, and twenty seven spp. were named probably as a result of rising in the quantity and specificity of identification methods in 2012 (Peleg et al., 2012). After the extensive phenotypic and genotypic analysis, the discovery of new spp. of *Acinetobacter* still occurs today (Nemec et al., 2015, 2016). In 2016 fifty one spp. have been accepted and named probably (Al Atrouni et al., 2016). Currently the genus is a complex and heterogeneous group of 55 species with valid names (Turner et al., 2018). the most

recent studies conducted by Elnar et al, in the first month of 2020, which he named *Acinetobacter pullorum* for strain B301^{T}, isolated from raw chicken meat in a local market in Korea. Currently, the *Acinetobacter* genus, consisting of 63 spp. according to the List of Prokaryotic names with Standing in Nomenclature, as published names *Acinetobacter calcoaceticus* the type species (Elnar et al., 2020). Spp. *A. calcoaceticus*, *A. baumannii*, *Acinetobacter* (GS) three, and (GS) thirteen TU are intimately connected. These spp. are referred to (*Acinetobacter calcoaceticus-Acinetobacter baumannii* (Acb) complex) which they cannot differentiate them from their phenotypic morphologies (Gerner-Smidt et al., 1991; Gerner-Smidt, 1992). More modern researches have also verified the connection of these spp. utilizing specialized genetic methods, for examples analyses of housekeeping gene sequences and whole genome analysis (Périchon et al., 2014; Touchon et al., 2014). However, in similar methods, (GS) three was classified as *A. pittii* while (GS) thirteen TU has been classified as *A. nosocomialis* (Nemec et al., 2011). Due to their similarity to these species, they added two (GS) and Nemec et al named (Close to thirteen TU), and (Between one and three). Recent research described the (Close to thirteen TU) (GS) *A. seifertii* (Nemec et al., 2015). In 2019, Nemec assumes that the name *Acinetobacter lwoffii* pertains to GS9 and not to GS8 as Bouvet and Grimont in 1986, delineated two similar taxa of the genus *Acinetobacter* called genospecies 8 and 9, they suggested the name *Acinetobacter lwoffii* for GS8, then Nemec said that these classes represent two species. And suggest the name *Acinetobacter pseudolwoffii* species for GS8 (Nemec et al., 2019). The standard biochemical tests cannot clearly differentiate members of the Acb complex from one another. As a consequence of diseases induced by members of this group have been linked to in various periods of history, which produced via *A. calcoaceticus*, *A. baumannii*, or the (*Acb*) complex. Meanwhile, the progress of detection methods has established that the *Acinetobacter baumannii* is the most commonly isolated spp. of this complex in human contamination (Peleg et al., 2008).

2.2. Epidemiology

Acinetobacter strains are widely derived from natural sources like soil, water, and sewage, and are also found in milk and dairy products, poultry, and frozen food (Bergogne-Berezin and Towner, 1996; Towner et al., 1991). Additionally, strains of this genus that have been isolated from vegetables are generally less associated with the human disease with differing averages which can extend to fifty one percent (Berlau et al., 1999; Houang et al., 2001). Generally *Acinetobacter* species and specifically *A. baumannii* were extracted from animal samples (Francey et al., 2000; Vaneechoutte et al., 2000), as well as in France homeless people were obtained for Acinetobacter infection from body lice (La Scola and Raoult, 2004). First *Acinetobacter* clinical isolate was isolated from the ICU in 1969 from Manchester Royal Infirmary in Manchester city-United Kingdom (Stirland et al., 1969). The appearance of these species in these different conditions led this group being regarded as unique in their instinct (Baumann, 1968; Fournier and Richet, 2006). Environmental wound contamination has also been investigated as soldiers wounded in the Afghanistan, Iraq and Vietnam wars had a high rate of *Acinetobacter baumannii* wound and blood infections (Hujer et al., 2005). *Acinetobacter* is a group of commonly found bacteria (germs) in the environment, such as in soil and water. Although there are several forms of infections, *Acinetobacter baumannii* is the most common cause in humans infections (CDC, 2019).

Acinetobacter species were classified as normal-microbiota in people who are in good health. The major spp. that identified to settle in the skin and mucous layers of people who do not suffer from disease are *A. lwoffii*, *A. junii*, *A. radioresistens, A. johnsonii*, *A. nosocomialis*, *A. pittii*, *A. variabilis*, and fewer range of *A. baumannii* (Berlau et al., 1999; Chu et al., 1999; Seifert et al., 1997). Furthermore, regular diversity in the skin settlement in catheters through the *Acinetobacter* species was developed. These species were diffuse more in the summer time in comparison to the winter time, (Chu et al., 1999; McDonald et al., 1999). *A. johnsonii*, *A. guillouiae*, and infrequently *A. baumannii* have recorded in healthy human stool specimens showing the exitance in

intestinal normal-flora of the bacteria mentioned above (Dijkshoorn et al., 2005; Nemec et al., 2010).

As a global pathogen, *Acinetobacter* was first reported to be a significant pathogen in the Korean war. This was verified during the Vietnam War, where it was the most prevalent gram-negative bacillus isolated from traumatic infections at lower extremities and the second most prevalent organism isolated from the blood (Villegas and Hartstein, 2003). And also soldiers from the Iraq and Afghanistan war were infected from hospital-acquired multidrug-resistant strains *Acinetobacter* (Towner, 2009). Overall the results indicate the wide distribution of *Acinetobacter* species especially *Acinetobacter baumannii*, and may provide insight into its potential natural habitat as well as providing a route for the dissemination into the hospital setting. The hospital environment serves as a suitable habitat for *Acinetobacter baumannii*, *Acinetobacter pittii* (formerly known as GS three) and *A. nosocomialis* (known as GS thirteen TU) (Lenie Dijkshoorn et al., 2007; Nemec et al., 2000; Peleg and Hooper, 2010). These species are able to persist for long periods of time up to several months on inanimate surfaces. In addition, the medical environment provides a larger number of hosts that could be infected by *Acinetobacter baumannii*. The moist conditions in hospitals, the room temperature along with the other devices can contaminate, all provide optimal survival and disseminated environments for *Acinetobacter baumannii*. (Nemec et al., 2000; Peleg and Hooper, 2010). *Acinetobacter calcoaceticus-Acinetobacter baumannii* (Acb) complex, as the 1970s this pathogen has been viewed as a commensal opportunist of negligible clinical significance and was regarded as significant originating from the hospital. It was assessed that forty-five thousand United States and one million worldwide instances of *Acinetobacter* contaminations happen every year. *A. baumannii* was stated to be the most significant clinical in over sixtiy spp. and Acb complex has also been extracted extensively from patients who diagnosed with pneumonia and bloodstream disorders. All drugs in areas have a major transient reductions in their susceptibility within Acb complex were evaluated. Findings for the strongly resistant to drugs along complex Acb levels were

maximum in Europe with sixty-six, followed by sixty-one in Latin America, fifty-six in Asia-Pacific, and North America with thirty-eight percent (Gales et al., 2019).

2.3. Pathogenesis and Pathogenic Mechanisms

The factors which make the *Acinetobacter* more pathogenic bacteria are drug resistance, community-acquired, and hospital-acquired especially ICUs infections. the reasons for this development to having those factors are the use of strongly new antibiotics in the clinical practice, instead of considering it as in the past saprophytes with low clinical importance. (Guardabassi et al., 1999). *Acinetobacter baumannii* was considered a worldwide nosocomial pathogen followed by *Acinetobacter lwoffii*, and *Acinetobacter haemolyticus*. *Acinetobacter* species disorders include bloodstream infections and ventilator-associated pneumonia which are common, and also include urinary tract infections, skin and wound infections, bacteremia, cholangitis, peritonitis, meningitis, ventriculitis, and infective endocarditis (Berlau et al., 1999; Jain and Danziger, 2004). The bacteria can be part of the normal flora in the skin and respiratory tract as harmless microbe. Disease acquire when hosts of the first line defense immune system are imperiled. Some researches were notified that the settling developed if the patient be at the hospital for a long period (Doughari et al., 2011). The changes of normal tissue construction of the gastric epithelium in gastrointestinal infections which lead to chronic gastritis. *Acinetobacter lwoffii* infections cause release of cytokines and raise gastrin concentrations which eventually improve the spread of gastric epithelium. Toll-like receptor, somatostatin, macrophage chemotactic protein, acid-secreting reactive oxygen species, macrophage inflammatory protein, and changes in the amount of gastric epithelial cells these cytokines secretion and the activation of antigen-presenting cells induces in chronic inflammation which can lead to peptic ulcers, gastritis, and more rarely gastric cancer (Richet and Fournier, 2006). Certain diseases associated with *Acinetobacter* species involve suppuration; brain, lung, and thyroid

abscesses can also develop into the secondary wound or surgical trauma infections, and purulent eye lesions (Dorsey et al., 2004).

Acinetobacter species are considered highly pathogens bacteria due to their resistance to many antimicrobial agents. They spread simply among patients and can survive in dry condition. Furthermore, to their multiple-day persistence in the environment that arose as essential opportunistic pathogens due to features that support their persistence in the hospital climate. In particular, the incidence of rapidly progressive community-acquired Acinetobacter pneumonia suggests this microorganism could be extremely pathogenic and cause serious infections (Bergogne-Berezin and Towner, 1996; Joly-Guillou, 2005). *Acinetobacter radioresistens* are highly resistant to drying conditions and can survive at 31 percent relative humidity for around of 157 days. Houang et al. (1998) demonstrated that *Acinetobacter baumannii* survives in dry conditions for thirty days and that *A. lwoffii* lasts up to 3 weeks. No differentiation is created between sporadic A. baumannii strain survival periods and epidemic strain (Braun, 2008). In these components, the pathogenic pathways were described include toxins, capsule and extracellular viscose compounds, enzymes, surface proteins and carbohydrates, and other small molecules. Gram (negative) bacteria like *Acinetobacter* having lipopolysaccharide (LPS) in their capsule which is a component of the outer membrane of the cell wall, composed from O polysaccharide, core, and lipid A. In human serum O polysaccharide is linked in resistance to the complement, and acts in synergy with capsular exopolysaccharide (Goel and Kapil, 2001; Braun, 2008). There are little researches on the pathogenic mechanisms of the *Acinetobacter* species. Eventhough *A. baumannii* is the species that were investigated among researchers, the development of infections remain unknown. The organism was not processed to generate toxins or cytolysins that capable to spread, and few virulence factors have been identified which were determined from comparative genomic studies between *Acinetobacter baumannii* and the environmental *Acinetobacter baylyi*. Virulence factors of *Acinetobacter* have identified genes involved in pilus biogenesis, iron uptake and metabolism, quorum sensing, and a type IV secretion system (Doughari et al., 2011).

2.4. Virulence Factors

Part of what makes the species *Acinetobacter* an effective germs and its capability to cause many virulences. *Acinetobacter baumannii* has a number of virulence pathways, including siderophore-mediated iron acquisition processes, biofilm production, adherence, and outer membrane protein activity, LPS, capsule forming, and quorum sensing (Peleg et al., 2012). *A. baumannii* is part of (ESKAPE) pathogen (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*), a class of highly antibiotic-resistant bacteria that are responsible for most infections from nosocomial diseases worldwide (Handal et al., 2017; Santajit and Indrawattana, 2016).

2.4.1. Cell Surface Structure and Enzyme

Gram-negative bacteria cell surface plays a major function within that metabolism among these species, along with transportation of substances into and out of the cell, contact, and sensing of the extracellular medium, and protecting against environmental stress (Silhavy et al., 2010).

Lipooligosaccharide (LOS)/Lipopolysaccharide (LPS)

Lipooligosaccharide also known as lipopolysaccharide, the main part of the outer membrane layer of several Gram (-) bacteria including Acinetobacter species. LPS consists of the endotoxic lipid A, a core oligosaccharide, and the O antigen which is a repeating sugar structure (Raetz and Whitfield, 2002). Throughout the core oligosaccharide LPS production is made through to lipid A ligand in the cytoplasm and whipped into the periplasmic area. The repeated subgroup of the O antigen formed independently on a transporter of undecaprenyl phosphate (Und-P), which will be dragged into the periplasm and ligated to the lipid A core by the WaaL ligase enzyme which considered as helping factor for O antigen (Whitfield et al., 1997; Liu et al., 2014; Stenutz et al., 2006). *Acinetobacter* species encode proteins based on their strain for either of two related specialty genes located in WaaL ligase orthologues, nevertheless,

these genes are placed in PglL that are oligosaccharyltransferases (OST) produced from *Neisseria meningitidis*, proposing that *Acinetobacter* species generate LOS but not LPS by OST that responsible for O-linked protein glycosylation. In every scenario, selective and unpredictable mutations of the genes resulting in the production of the LOS core oligosaccharide has shown that this portion contributes greatly to the persistence and pathogenicity of *Acinetobacter* (Iwashkiw et al., 2012; Harding et al., 2015; Schulz et al., 2013; Kenyon and Hall, 2013; McQueary et al., 2012; Luke et al., 2010; Lin et al., 2012).

Capsule

Like several other organisms, *Acinetobacter* spp. contain an extracellular capsule and maintains a thick shield against foreign invaders, such as complementary killing cells (Russo et al., 2010). It has recently been reported that the development of capsules could be increased by the existence of sub-inhibitory antibiotic quantities that promote resistance to complementary killing cells and lead to systemic infection by cause hyper-virulent morphology in a mouse pattern (Geisinger and Isberg, 2015). The two-component Biofilm Regulating System (BfmRS) mentor numerous essential virulence in *Acinetobacter* also can mentor the phenotype of the capsule (Tomaras et al., 2008). *Acinetobacter* species also present poly-beta-1-6-N-acetylglucosamine (PNAG) polysaccharide connected to a surface that is essential in increasing the virulence and biofilm producing (Choi et al., 2009).

Pili

In 1975 Henrichsen has identified that *Acinetobacter calcoaceticus* strains which shown that the surface fimbrial structures twitching motility. Pili is filamentous bacterial surface appendages which interacts with the spp. and their environment (Nenrichsen, 1975; Hentuchsen and Blom, 1975). The positions pili have a major role in the physiology and pathobiology of pathogenic *Acinetobacter* spp.. All sequenced pathogenic *Acinetobacter* species have been described with a chaperone/usher pili system which are proteins called Csu pili (Tomaras et al., 2003). And those are needed

for the development or stabilization of *Acinetobacter baumannii* in biofilms but have not been observed to involve adherence to humans tissue (de Breij et al., 2009). It has also been shown that medically important species of *Acinetobacter* develop type IV pili (TFP), active bacterial surface appendages established to influence twitching motility, horizontal gene transfer and the development of biofilms (Burrows, 2012; Harding et al., 2013).

Protein secretion

The current study has investigated numerous pathways used by *Acinetobacter* to release proteins, like outer membrane vesicles, type II secretion, autotransporters, type VI secretion. The secretion of these proteins in *Acinetobacter* has indicated distinct processes such as positions in horizontal gene transmission, antibiotic susceptibility, and pathogenicity (Weber et al., 2017).

Outer Membrane Proteins (OMPs)

OmpA is the most common virulence factor in *Acinetobacter baumannii* OMP. OmpA generates an 8-stranded β barrel in the outer membrane, by a two-nm pore diameter and a C-terminal periplasmic globular length. Interestingly, OmpA was been advertised as an important vaccine production target (Ansari et al., 2018; Choi et al., 2008; Iyer et al., 2017).

Enzymatic Activities in *Acinetobacter*

Urease, esterase processes, other amino-peptidases, and acid phosphatase can induce in *Acinetobacter baumannii* pathogenicity. Higher esterase activity is known to hydrolyzing short-chain fatty acids in ester interactions, which can lead to lipid tissue degradation (Braun, 2008). The activity of urease, that varies within *Acinetobacter* strains, allows the bacteria to settle the inflammatory hypochlorhydric or achlorhydric stomach (Rathinavelu et al., 2003).

Phospholipases are significant human pathogens and *Acinetobacter* reproduces mainly C and D enzymes, distinguished by its affinity for cleavage location (Fiester et al., 2016; Schmiel and Miller, 1999).

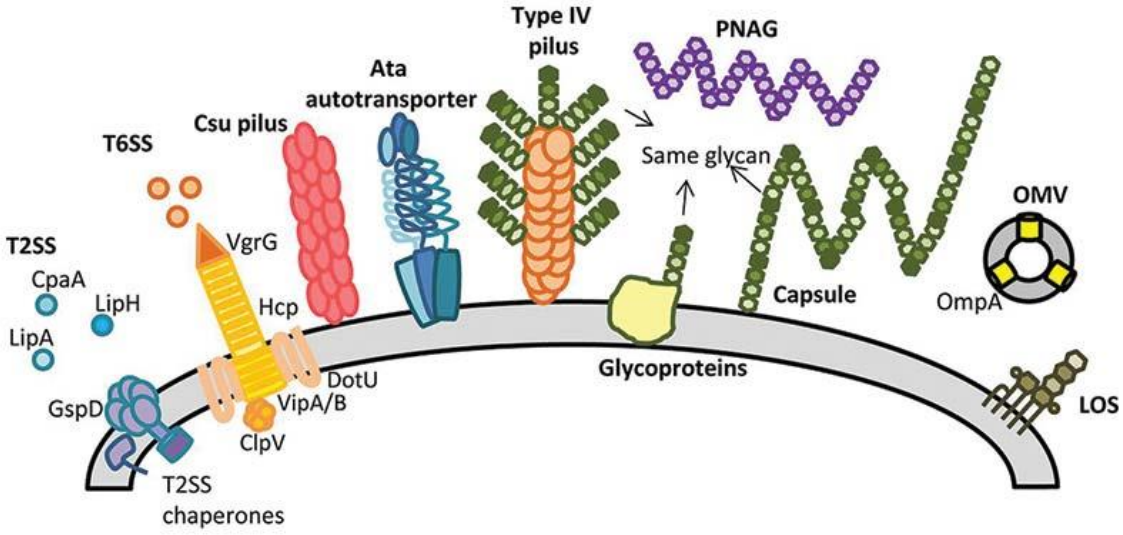


Figure 1. Cell surface components and mechanisms of secretion found in the genus *Acinetobacter* (Weber et al., 2016).

2.4.2. Toxic Slime Polysaccharides

In *Acinetobacter* species toxic slime polysaccharides were also identified. They are commonly formed during the exponential growth process and compose of the D-glucuronic acid, D-mannose, L-ramnose, and D-glucose building blocks. Toxic slime polysaccharides are extremely harmful to neutrophils, frustrating their movement and phagocytosis however without interfering with the host immune system (Doughari et al., 2011).

2.4.3. Verotoxins

The development of verotoxins from *Acinetobacter haemolyticus* was first reported at *Acinetobacter* species. Verotoxins are associated with bloody diarrhea, the

pathogenicity, basic structure, and chemical properties of the toxins similar to those verotoxins of *Escherichia coli*, *Shigella dysenteriae*, and many enteric bacteria (Doughari et al., 2009; Lambert et al., 1993). Vero-toxins can be categorized into two classes of antigens: vtx-1 and vtx-2. The toxins connect to a specific protein subfamily, the RNA N-glycosidases that directly target the machinery of the cell ribosome, inhibiting protein synthesis (Lambert et al., 1993).

2.4.4. Iron Recovery Mechanisms

Another factor associated with *Acinetobacter* virulence siderophore synthesis, known as low molecular weight compounds, the process of changing polymeric ferric oxyhydroxides through active iron chelates created by bacteria thriving in low iron doses (Braun, 2008). Siderophores are iron-binding structural proteins host that necessary to iron nutrition in bacteria although the protective mechanism for pathogenic bacteria is the removal of free concentrations of extracellular iron by iron-binding proteins such as lactoferrin or through transfer (Yu et al., 2005). Bacterial siderophores are called (Aerobactins), and *Acinetobacter* siderophores are called (Acinetobactins) and consist basically of the amine histamine formed by histidine decarboxylation (Mihara et al., 2004). Iron entry into the bacterial cell is therefore monitored by a ferric controller that absorbs protein and acts as a transformation repressor to stimulate siderophore formulation or destruction (Vallenet et al., 2008).

2.4.5. Mechanism of Adhesion and Damage to Tissue

Bacterial adherence is typically a complex mechanism involving compounds on the cell surface of the bacteria, and complementary receptors on the host cell surface. The bacterial adhesives comprise fimbriae (pili), capsular polysaccharides or cell wall components (Braun, 2008). Adhesion is an essential and initial stage in infections with *Acinetobacter baumannii*. *Acinetobacter baumannii* can then invade host cells such as human lung, laryngeal, and cervical epithelial cells after adhesion (Parra-Millán et al., 2018). *Acinetobacter baumannii* is capable of reaching inside host cells and persisting. It adheres to host cells first, then invades and translocates into nucleus. It spreads

throughout the bloodstream and tissues after killing of host cells (Nie et al., 2020). Interestingly, *Acinetobacter baumannii* able to persist within host cells, even so, there is no replication was reported in vitro and in vivo results have shown that *Acinetobacter baumannii* causes death of host cells and spreads in tissues and bloodstream. Though the spread to deeper tissues leads to invasive diseases (Parra-Millán et al., 2018). OmpA functions in the adhesion and tissue damage process for *Acinetobacter baumannii* separated into the upper panel and lower panel. In the upper panel, once interacting with the epithelial cells, the bacteria produce (OmpA) into the epithelial cells are, (OmpA) capable of translocating into nucleus and mitochondria and activating mitochondria to release cytochrome c which is a heme protein. Cytochrome c then facilitates the translocation of apoptosis-inducing factor (AIF) into the nucleus and eventually induces apoptosis of epithelial cells. In the lower panel, (OmpA) increases nitric oxide synthase (iNOS) production and Toll-like receptor 2 (TLR2) surface expression in epithelial cells, both of which leads to host cell apoptosis (Nie et al., 2020).

2.4.6. Quorum Sensing (QS)

Bacteria stay in close contact with other bacteria and the eukaryotic hosts within the community. Spp. of bacteria have to continue to monitor and communicate among neighbors. In a process called quorum sensing, bacteria develop autoinducers, hormone-like molecules, as signals for sensing cell density and activating adaptations. Autoinducers function via linking on regulatory transcriptional proteins, activating, and controlling gene activity inside this microbe. *Acinetobacter* produces acyl-homoserine lactones (AHL) as a signaling molecule for the contact between interspecies and intraspecies, the same as Gram-negative rods organisms. Examinations have combined (AHL) interfered Quorum Sensing with phenotypes that serve the AHL-producing community, virulence factors, motility, bioemulsan production, bioluminescence, biofilm formation, nodulation, plasmid transfer, and antibiotic production (Asif et al., 2018; Bhargava et al., 2010; Saipriya et al., 2020).

2.4.7. Biofilm

The biofilm is constructed in a tertiary model in which the bacteria are in direct contact to each and embedded in a matrix of extracellular polymeric substances (EPS), that may incorporate exopolysaccharides, nucleic acids, proteins, and other macromolecules (Barraud et al., 2015; Branda et al., 2005). Biofilm is a collection of separate bacterial or combined cells that attach to abiotic or biotic plates (Hoiby et al., 2010). primarily factors behind the formation of bacterial biofilms are natural growth pattern for certain species, shield from foreign harmful conditions, preferential colonization under conditions rich in nutrients, and cooperative effects as part of the community (Hall-Stoodley et al., 2004; Jefferson, 2004). Biofilms were found in rivers on rocks and pebbles, contaminated water beds, ponds, waste and water tanks, military marine construction structures, boat hulls, etc. Microbial settling of human cells, such as heart plugs, teeth veneer, middle ear, injuries, and surgical instruments, is of huge importance to the health. Those clinical biofilms are liable for sixty-five to eighty percent of human infection, which could result in morbidity and death. Within these biofilms, bacterial cells produce phenotypes that differs from native cells and is more resistant to current drugs, ultraviolet light, dry condition, extreme pH and immune system (Singh et al., 2016). *Acinetobacter baumannii* has developed as the most widespread pathogenic species in hospital-acquired infections, among the *Acinetobacter* genus. And can withstand nutrient-limited surfaces, even under dry and in harsh hospital environments several days (Martí et al., 2011). Also, biofilms form an atmosphere of a huge amount of germs, therefore promote the increasing of the exchange of DNA and RNA which can carry the genes of virulence and part of its resistance. In addition, *A. baumannii* strains could live in biofilms and can communicate with each others within quorum sensing (Aminov, 2011; Niu et al., 2008). Within this connection, *A. baumannii* can produce the evolution of the slow persister cell in deep surfaces which are permissive to antimicrobial agents (Bhargava et al., 2014). These persistent cells in the biofilm may serve as a source for the bacterial species to be refreshed and are shielded from antibiotic damage. The newly divided cells may still include genetic material from

their environment because these bacteria may potentially be resistant to antimicrobials through this phase. Furthermore the cells of bacterial on the top of biofilm surface have been found capable of separating themselves from them (Derlon et al., 2013). A research by Badmasti et al. in 2015 revealed that resistance antibiotic and biofilm evolution rely on the expression of genes variety. In particular, they theorized that recognizing the relations between these two incidents have a threaded ability to understand the processes of *A. baumannii* persistence in hospitals and its attachment of medical devices like catheters and ventilator machine (Badmasti et al., 2015).

2.4.8. Motility

For several different genera, for example, bacterial motility is strongly connected to the capacity of an organism to produce disease; in the case of *Pseudomonas aeruginosa*, the flagellum acts as the main generator of bacteria, which also induces in virulence. Similarly, Hypermotility of *Acinetobacter baumannii* has been related to progress infectivity in the Caenorhabditis elegans disorder and vice versa, the attenuated phenotype was seen by a defective mutant in motility. (Eijkelkamp et al., 2013; Pérez-Varela et al., 2017). Furthermore, recent epidemiological research on *Acinetobacter baumannii* of clinical tests showed that blood specimen were more motile than sputum one when measured, suggesting that motion in different positions of the body can provide an advantage in movement (Vijayakumar et al., 2016). Paradoxically, *Acinetobacter* in general were considered to non-motile. *Acinetobacter baumannii* and *Acinetobacter nosocomialis* however, are able to develop two separate modes of microbial locomotion which are surface motility and twitching motility. About twitching motility in *Acinetobacter baumannii* and *Acinetobacter nosocomialis* is relied on the full function of type IV pili for frequent series owing to expansion and shrinkage to draws cells of bacteria onwards (Harding et al., 2018).

2.5. Antibiotic Sensitivity and Antibiotic Resistance Mechanisms

Antimicrobial resistance is a phenomenon recognized immediately certain substances detected from long time ago. Studies suggest the trend is old and occurred

during the period of time preceding the invention of antibiotics (D'Costa et al., 2011). So about pathways of resistance for *Acinetobacter*, there are various pathways that *Acinetobacter* adopts to avoid antibiotic devastation. Which it is enzyme-mediated degradation (beta-lactamases), genetic manipulation (mutations, addition or removal of a gene, upregulation or downregulation of gene expression), and efflux pumps (Martínez-Guitián et al., 2016).

Acinetobacter baumannii causes severe infections of the nosocomial type. Because of its severe problems with drug resistance, the complex mechanism for drug resistance, and fast antibiotic adaptation. So there are recent studies that show how to make this species sensitive to the antibiotics through some mechanisms like; in vitro studies found that the combined use of serum Anti-*Acinetobacter baumannii* outer membrane vesicles (OMV) and quinolone antibiotics significantly enhanced the bacteria sensitivity to these antibiotics. The use of serum Anti-*Acinetobacter baumannii* outer membrane vesicles and levofloxacin in a pneumonia model increased the sensitivity of levofloxacin, and bacterial amounts in the lung and spleen limited due to antibiotic or antibody concentrations. This approach has greatly decreased the penetration of inflammatory cells by the lung and accumulation of inflammatory cytokines. Efflux pump when conjunction with antibiotics, the Efflux pump antibodies increase the sensitivity of drug-resistant bacteria to antibiotics. Nevertheless, when antibodies damage an essential outer membrane protein, bacteria can control the expression of other proteins on the outer membrane to form a drug-resistant outer membrane protein interaction and allow survival (Al-Hamad et al., 2011; Zhang et al., 2019).

2.5.1. Resistance of Beta-Lactam Antibiotics

Beta lactams are antimicrobial agents which contains a variety of bactericidal substrates commonly utilized in curing bacterial infection. These drugs are classified into (penicillin, cephalosporin, monobactam, and carbapenem) (Dalhoff et al., 2006; Elander, 2003). Each of these antimicrobials has a beta lactam ring in common and bounding to D-alanyl-d-alanine (DD)-transpeptidases with various affinities blocks the

development of the bacterial peptidoglycan layer commonly known as penicillin-binding proteins (PBPs) (Fisher et al., 2005). In addition, this blocks the cross-linkage of the immature peptidoglycan layer that prevents the formation of cell walls. In fact, some β-lactamase restricts were joined with β-lactam because of the production of enzymes that hydrolyze beta-lactams. Amoxicillin-clavulanic acid, ampicillin-sulbactam, and piperacillin-tazobactam are the most common combinations (Drawz & Bonomo, 2010). Beta-lactamases, which are found among Gram (-) bacteria that beta-lactams are hydrolyzed by splitting the amide link of the beta-lactam ring were through several enzymes that hydrolyze antibiotics. β-lactamases have been categorized to Classes which are (A, B, C, and D) by ambler sorting built on the molecular composition. The Extended Spectrum Beta Lactamases (ESBLs) hydrolysis a wide variety of beta lactams within this group of enzymes, and carbapenemases can hydrolyzing carbapenem (Ambler, 1980; Weldhagen et al., 2003; Xia et al., 2016). Beta-lactam antibiotic resistance is mediated by enhanced beta-lactamases degradation, alteration of penicillin-binding proteins, modulation of the outer porins membrane for lowered permeability, and exclusion of antibiotics cell via efflux pumps. Beta-lactamase AmpC cephalosporinase (class C) is more prevalent in *A.baumannii* (Davies and Davies, 2010; Eliopoulos et al., 2008). These were mediated via (bla) gene and include penicillin resistance, cephalosporins with a narrow-spectrum, and cephalosporins with an extended-spectrum. Certain beta-lactamases class A include which are (PER-1, VEb-1, CTX-M, TEM, SHV), Metallo Beta-lactamases for Class B beta-lactamases which are (MBLs; IMP, SIM, VIM), and OXA for Class D beta-lactamases (Perez et al., 2007). Carbapenems antibiotic which is the treatment of choice among this bacteria, the acquired OXA-type carbapenemases are the backbone distraction of these antibiotics, followed by MBLs (Thomson & Bonomo, 2005). OXA23, OXA24, and OXA58 that have been primarily liable to recession carbapenem were plasmids that encode carbapenemases. The cooperation of OXA23 and an MBL NDM-1, identified as an illusion in the antibiotic resistance background (Karthikeyan et al., 2010). OXA are chromosomal interceded including OXA25, OXA26, and OXA40. The reduced entry of drugs by outer membrane proteins (OMPs) or porins, and modification of penicillin-

binding proteins (PBPs), are involved in beta-lactam resistance (Morán-Barrio et al., 2017). The involvement of efflux pumps provides resistance to several antibiotic classes. Six families of efflux pumps have been described, including resistance nodulation cell division family, ATP binding cassette (ABC) family, multidrug toxic compound extrusion family, major facilitator superfamily, small multidrug resistance superfamily, and recently discovered proteobacterial antimicrobial compound efflux family (Buckner et al., 2016). AdeABC efflux pump featured well in *A. baumannii* which is a member of the family resistance nodulation division cell (RND), mediating the resistance to numerous types of drugs (chloramphenicol, fluoroquinolones, cefotaxime, aminoglycosides, and erythromycin). AdeABC over-expression gives resistance to carbapenems and other types of efflux pumps for the remaining families (Yoon et al., 2016).

2.5.2. Resistance of Aminoglycosides

Aminoglycoside Modifying Enzymes (AMEs) express the aminoglycosides resistance by enzymatically modifying the amino or hydroxyl groups of these antimicrobials. *Acinetobacter* has identified all categories among enzymes derived from aminoglycosides (acetylases, adenylases, methyltransferases, and phosphotransferases). Some several pathways required intolerance to aminoglycosides are lowered drug import and modulation of the intended ribosomal protein (Shrestha et al., 2016; Vakulenko and Mobashery, 2003).

2.5.3. Resistance of Quinolone

Mutations in genes gyrA and parC are the key pathways for quinolone resistance, which leads to chromosomal shifts in DNA gyrase and topoisomerase IV and lowers drug sensitivity afterward (Ugolotti et al., 2016). On the other hand, drug influx and efflux mechanism mediated by chromosomal DNA that has been decreased medication-influx production of OMPs and enhanced efflux protein expression this contributes to effective deportation of drugs thus also induce in resistance to quinolones (Charrier et al., 2016). Determinants of plasmid-encoded resistance to quinolones qnrA, qnrB and

qnrS were noted in *A. baumannii* which defends DNA via blocking DNA-gyrase and topoisomerase binding of quinolones (Ling et al., 2016; Yang et al., 2016).

2.5.4. Resistance of Tetracycline

Tetracycline antibiotics are well known for their broad spectrum of activity, minocycline and doxycycline are two tetracyclines, used to treat *A. baumannii* infections. Their important mechanisms have effective arbitration levels towards Carbapenem-Resistant *A. baumannii* (CRAB) disorders with a relatively weak toxic side effect (Chan et al., 2010; Grossman, 2016; Wood et al., 2003). These drugs link to the 30s ribosomal subunit and prohibit aminoacyl-tRNA from being integrated into the A location and thereby suppress protein production. Nevertheless, In *A. baumannii*, widespread efflux pumps and Ribosomal Protection Proteins (RPPs) shield this species from these antimicrobial activities and limit its medicinal use (Connell et al., 2003; Doi et al., 2015).

2.5.5. Resistance of Colistin

The method of antimicrobial activities with colistin remains mysterious but the process is currently understood that colistin binds lipopolysaccharide of Gram (-) bacteria, allowing bulge of the outer membrane, eventually interferes with the phospholipid bilayer, resulting in an osmotic disequilibrium that corresponds to cell death, via a self-promoting absorption procedure (Bader et al., 2003; Zavascki et al., 2007). The process of *A. baumannii* colistin resistance is basically localized in lipid A modification or lipopolysaccharide degradation. Comparison of DNA sequences of the PmrA/PmrB two-component scheme (TCS) in *A. baumannii* colistin-resistant and sensitivity and finding mutations in the resistant strains (pmrA/pmrB), contributing to the theory that PmrAB controls *A. baumannii* susceptibility to colistin. Throughout the mechanism of colistin resistance is known basically localized in lipid A modification or lipopolysaccharide degradation, In a significant number of colistin-resistant *A. baumannii* isolates, pmrA and pmrB mutations were shown to conformational changes induce pmrA expression, therefore nature regulating the gene expression of pmrCAB

and passing phosphoethanolamine (pEtN) to lipid A. In this factors allowed to modify lipid A on the site of the 4′-phosphate group (Adams et al., 2009; Olaitan et al., 2014). Related study Explained that two-component device PmrA and PmrB would concurrently manage the synthesis of NaxD deacetylase and lipid A alteration by deacetylated β-galactosamine resulting in a loss of colistin responsivity (Chin et al., 2015). The LpxACD genes were responsible for coding the enzymes that induce in lipid A synthesis in the first three stages. Blocking lpxA or lpxC prevents colistin from exerting action and resistance (Moffatt et al., 2010, 2011). Nowadays, studies have shown that two publish mutations, pmrA (I13 M) and pmrB (Q270P), are implicated in resistance of *A. baumannii* colistin and the pmrA (P102R)-mediated colistin resistance is further improved by miaA. These findings will help to increase our knowledge about the colistin resistance mechanism in *A. baumannii* (Sun et al., 2020).

2.6. Treatment

Throughout the 1970s, the majority of antimicrobials available were widely used in the treatment of *Acinetobacter* species infections, increased rates of resistance to different antimicrobials started as early, where resistance towards imipenem has been noted, till the early 1990s. Also, there is resistance to other medical drugs over the same strains because of the variety of resistance mechanisms within *A. baumannii* (Bergogne-Berezin and Towner, 1996; Doi et al., 2015). However, due to the resistance of many antibiotics, the isolated microbes were classified as follows; where the microbe is not responsive to at minimum one antibiotic agent in three or more groups named as Multi-Drug Resistant (MDR); where they are not responsive to at minimum one agent in any but two or less classes of antibiotics named as Extensively-Drug Resistant (XDR); and where they are not responsive to all antibiotic groups named as Pan-Drug Resistant (PDR) (Magiorakos et al., 2012).

A number of recent quinolones such as levofloxacin, ciprofloxacin, moxifloxacin, ofloxacin, and gemifloxacin were formed for the treatment of MDR pathogens (Blair et al., 2015; Xiaobing Jiang et al., 2014). Resistance to new antibiotics is rapidly rising,

therefor new therapeutic approaches methods for treating MDR pathogens are required. Researches have concentrated on combination treatments though new single-drug production is expensive and time-consuming (Djeribi et al., 2012).

First-line agents for susceptible organisms; when infections are caused by *Acinetobacter* isolates susceptible to antibiotics, several therapeutic options may be obtainable, including a broad-spectrum cephalosporin (cefepime or ceftazidime), carbapenem (such as meropenem, imipenem, or doripenem) or a combination of beta-lactam/beta-lactamase inhibitors (such as sulbactam). Dosing is summarized separately in Table 1 (Dalfino et al., 2012; Plachouras et al., 2009).

Table1: Systemic antibiotics for treatment of *Acinetobacter* infection in adults with normal renal function.

Drug	Dose
Ceftazidime	2 g every 8 hrs (through venous)
Cefepime	2 g every 8 hrs (through venous)
Ampicillin-sulbactam*	3 g every 6 hrs (through venous)
Imipenem-cilastatin	0,5 to 1 g every 6 hrs to 1 g every 8 hrs (through venous)
Meropenem	1 to 2 g every 8 hrs (through venous)
Doripenem	0,5 g every 8 hrs (through venous)
Gentamicin	1 to 2,5 mg/kg every 8 to 12 hrs or 7 mg/kg every (one to two day) depending on creatinine clearance (through venous)
Tobramycin	1 to 2,5 mg/kg every 8 to 12 hrs or 7 mg/kg every (one to two day) depending on creatinine clearance (through venous)
Amikacin	5 to 7,5 mg/kg every 8 hrs or 15 mg/kg every (one to two day) depending on creatinine clearance (through venous)
Ciprofloxacin	400 mg every 8 hrs (through venous)
Colistin	2,5 to 5 mg/kg/day as **colistin base** in two to four divided doses (through venous)
Polymyxin B	25 000 units/kg (2,5 mg/kg) loading dose followed by 12 500 units/kg (1,25 mg/kg) every 12 hrs (through venous)

Minocycline	200 mg single dose, then 100 mg every 12 hrs (through venous)
Tigecycline	100 mg single dose, followed by 50 mg every 12 hrs, 100 mg every 12 hours in serious infections (through venous)
Ampicillin-sulbactam	Higher doses of ampicillin-sulbactam (eg, up to 3 g every 4 hrs)
Aminoglycosides and fluoroquinolones combination with another agent (colistin)	300 mg dose, then 150 mg every 12 hrs for treatment of sepsis due to multidrug-resistant Acinetobacter infection

In vitro and *in vivo* experiments have demonstrated that drug combinations if practiced upon a specimen of drug-resistant *A. baumannii*, may be synergistic and extremely bactericidal. These synergistic combinations usually require two or three separate antibiotic classes. Many experiments have already shown through the insertion of such antimicrobials especially chimeric peptides lead to enhanced the efficacy of medicines (Gopal et al., 2014). The advantages of amalgamation therapies provide a wide range of distribution, blocking of resistance, and synergistic effects around different antimicrobials (Lutsar et al., 2014). Recent mixture therapies studies have focused mainly on polymyxins, sulbactam, tigecycline, and rifampin or carbapenems, to sum up the vast *in vitro* and *in vivo*. Polymyxins were widely practiced as a substance enclosed under pressure and able to be released as a fine spray to treat *A. baumannii*-associated ventilator pneumonia, amidst lower nephrotoxicity than anticipated (Gales et al., 2006; Urban et al., 2001). Colistin used in 2 types: colistin sulfate for oral and topical use and colistin sulfomethate sodium for parenteral use, the others were inactive or (prodrug) parenteral treatment recommended due to their lower toxic activity. *A. baumannii* strains are susceptible to human toxic polymyxins and thus treatments that incorporate polymyxins will add for several drugs had to mitigate its adverse actions and diminish the dose, currently, illness in humans among *A. baumannii* this type of treating were described for this situation (Falagas et al., 2010). The combination of colistin with carbapenem, rifampicin, tigecycline, and other antibiotics in opposition to *A. baumannii* was described as the treatment of choice *in vitro*. Furthermore, the in vivo findings

recorded for solid organ transplant patients that are settled or gets their infection in (XDR) *A. baumannii* indicate for combination of colistin and carbapenem increases therapeutic reaction and keep going alive matching with the additional therapies such as colistin-tigetycline also can even restrict colistin resistance progressing (Shields et al., 2012). Combination of colistin and rifampicin also demonstrated improved clinical outcomes in the treatment of ventilator-associated pneumonia. Nevertheless, thirty day mortality does not decreased through applying rifampicin colistin while *A. baumannii* mortality levels increased (Aydemir et al., 2013; Durante-Mangoni et al., 2013).

Studies initially concentrated in combination therapy approaches along with minocycline-tigetycline, colistin-(tigetycline or rifampin), and mixed polymyxin-B medication. Most of these options show minimal benefits when treating hospital infections, leading eventually to an evolutionary pressure that raises the resistance rate of bacteria. Consequently, combination treatment in hospital environments may not be possible in the long term since the *A. baumannii* strains did not respond nearly to every antibiotic category. It should therefore be researched in accordance with the "post-antibiotic period" and the application of advanced methods to manage MDR*AB* propagation must be emphasized. The most advanced treatments, such as modern antimicrobial peptides, CRISPR Cas method (Clustered frequently interspaced short palindromic repeats), and phage therapy have been developed to inhibit MDRAB strains from the spread (Adams et al., 2009; D'Onofrio et al., 2020; Menegucci et al., 2019; Vrancianu et al., 2020).

Sulbactam has demonstrated the highest inherent bactericidal effectiveness towards *A. baumannii* isolates among the β-lactamase inhibitors. Clinical testing findings have recorded the effectiveness of sulbactam in light to critical for *A. baumannii* infections and effectively used sulbactam to treat MDR-*Ab* related infections which were meningitis, pneumonia, and bacteremia (Temocin et al., 2015).

In patients, sulbactam with ampicillin and carbapenems were used however bacterial resistance was risen compromising its usefulness. New research utilized tigecycline

towards illnesses with XDR *A. baumannii* examined one hundred twenty patients cured by imipenem and sulbactam (Lee et al., 2013). However if the dose is minimized in combination treatments, extended usage of antibiotics will disrupt the usual gastrointestinal microflora, reduce the original protection pathways given by the colon's microbial exosystemic and make the host susceptible to symbiotic microorganisms or nosocomial microbes (Rafii et al., 2008; Shin and Park, 2017).

3. MATERIALS AND METHODS

3.1. Collecting Sample

Between January 2012 and August 2020, ninety three (93) *Acinetobacter baumannii* complex strains isolated from different clinical samples in the Microbiology Laboratory of Near East University Hospital were included in this study retrospectively. Repeated strains of the same patient were exluded from the study. The isolated strains were kept in bacteria storage tubes (OR-BAK, Ankara, Turkey) until they were used at -80°C.

3.2. Identification and Antibiotic Susceptibility Test (AST)

Bacterial identification and ASTs were performed by full automated system Phoenix 100 (Becton Dickinson, Sparks, MD, USA) in line with the manufacturer's recommendations (0,45-0,55 McFarland) and results were evaluated according to the EUCAST (European Committee on Antimicrobial Susceptibility Testing) criteria.

3.3. Isolation and Storage

Storage tubes which were removed from -80°C to stimulate *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex strains were passaged into Brain Heart Infusion broth (BHI) (Becton Dickinson, Sparks, MD 211 52 USA) after reaching room temperature and incubated for 24 hours at 35°C. Then, each strains were cultured on Blood agar (Merck, KgaA, Germany) and Eosine Methilene Blue (EMB) agar (Becton Dickinson, Sparks, MD 211 52 USA) and incubated for 24-48 hours at 35°C again.

3.4. Double-Disc Synergy Test (DDST)

DDST is the test designed to detect ESBL production in bacteria. It was tested according to the EUCAST guidelines, and the bacterial suspension was prepared in accordance to the manufacturer's standard density (0,45-0,55 McFarland) then spread on Mueller-Hinton Agar (MHA) (Merck, KgaA, Germany) plate. Disks containing cephalosporins ((ceftazidime (CAZ), and/or ceftriaxone (CRO) or cefotaxime

(CTX), or cefpodoxime (POD)), and monocyclic beta-lactam aztreonam (ATM)) are placed to plates to a disk contain clavulanic acid (amoxicillin- clavulanic acid (AMC 20/10 μg)) in the center positioned at a distance of 30 mm (center to center). After incubation at 35°C overnight, the expansion of the inhibition zone around the cephalosporin or ATM towards the AMC disc, or the presence of a synergy area in which bacteria reproduce indicate the presence of ESBL (Gülay, 2004; Xiaofei Jiang et al., 2006). In this study DDST Disks containing cephalosporins (cefoxitin FOX 30 μg, ceftazidime CAZ 30 μg, cefepime FEP 30 μg) and (aztreonam ATM 30 μg) are placed to plates to a disk contain clavulanic acid (amoxicillin- clavulanic acid AMC 20/10 μg), NCTC 13353 strain of *Escherichia coli* was indicated as positive ESBL control in this method.

3.5. Congo Red Agar (CRA)

Biofilm formation was studied through the cultivation of all *Acinetobacter* clinical isolate strains on the reported Modified Congo Red Agar (MCRA) consisting of 0,4 g of Congo red dye (Alfa Aesar, ThermoFisher GmbH, Erienbachweg 2, 768 70 Kandel, Germany), 10 g of glucose (Merck, KgaA, Germany), and Blood Agar Base as nutrient agar. The dye was prepared in 100 ml of distilled water then autoclave at 121°C for 15 minutes, the glucose and the Blood Agar Base (Merck, KgaA, Germany) were dissolved in 900 ml of distilled water then autoclave at 121℃ for 15 minutes, then the dye was applied to the Blood Agar Base and the glucose then was blended well before infused into the plates. The color red has been described as negative biofilm, Black and Strong Black have been identified for positive formation of biofilms (Mariana et al., 2009). ATCC 6538 *Staphylococcus aureus* was indicate a positive biofilm control in this method.

3.6. Statistical Analysis

Qualitative and quantitative data values along with the percentage and mean ± standard deviation (SD) is represented as frequency. All statistical analyses were done using Statistical Package for the Social Sciences (SPSS) Version 25,0 (SPSS Inc. Chicago, IL, USA). Pearson Chi-square test is tested as appropriate on the association between two or more variables. Pictorial explanations of the major results of the study were rendered using an appropriate statistical graph. A $p<0,05$ was deemed significant.

4. RESULTS

4.1 Specimens Distribution

In this study out of 93 specimens, 62,4% (n: 58) were isolated from males and 37,6% (n: 35) from females as shown in Table 2 and Figure 2. The mean and standard deviation (minimum-maximum) of age in this study was found 67,39±16,99 (between 15-95). According to the age categories of participant patients, old adults with 88,2% (n: 82) were the most age category that infected samples collected from, the distribution of their ages was as shown in Table 3. And about the types of specimens that collected from, there are several different types, the majority were aspirate specimens 48,4% (n: 45) as shown in Table 4. Forty one 44,1% and n: 41 specimens were isolated from the general intensive care unit department among the distribution of specimens in all departments that collected from as shown in Table 5 and Figure 3.

Table 2. Distribution of gender

	Frequency	Percent (%)
Male	58	62,37
Female	35	37,63
Total	93	100,0

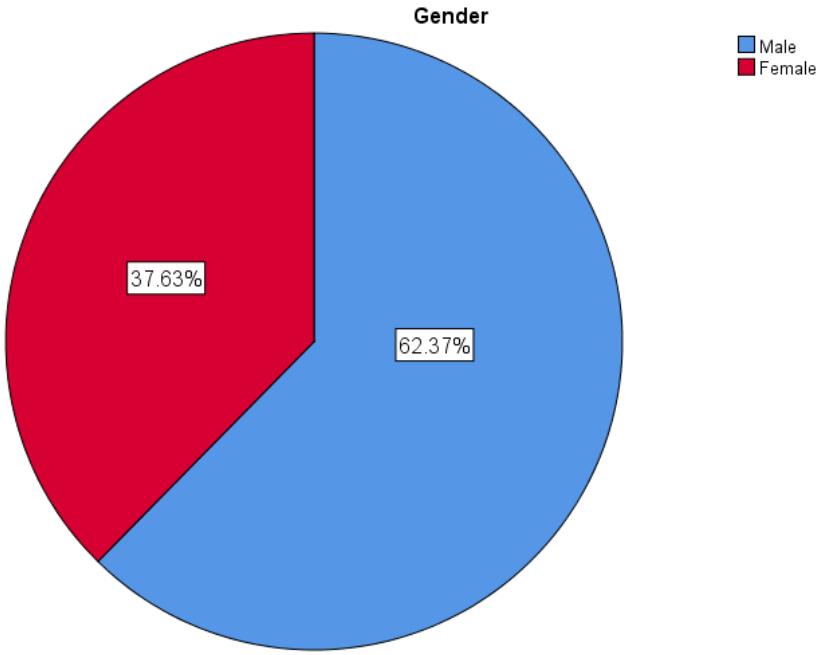


Figure 2. Distribution of gender

Table 3. Distribution of age category

	Frequency	Percent (%)
Child (3-15)	1	1,1
Young Adults (16-30)	5	5,4
Middle-Age Adults (31-44)	5	5,4
Old Adults (≥45)	82	88,2
Total	93	100,0

Table 4. Distribution of sample type

	Frequency	Percent
BAL	3	3,2
Aspirate	45	48,4
Blood	1	1,1
Sputum	18	19,4
Urine	18	19,4
CSF	1	1,1
Catheter	4	4,3
Wound	3	3,2
Total	93	100,0

Table 5. The distribution of the specimens among different hospital departments

	Frequency	Percent
Chest Diseases and Allergy	18	19,4
Geriatrics	2	2,2
Infectious Disease	1	1,1
Internal Medicine	3	3,2
Oncology	1	1,1
Pediatrics	1	1,1
Urology	1	1,1
Neurosurgery	4	4,3
Physiotherapy	1	1,1
Neurology	2	2,2
Orthopedics and Traumatology	5	5,4
Cardiology	7	7,5
Ear, Nose and Throat	1	1,1
Brain Surgery	5	5,4
General Intensive Care	41	44,1
Total	93	100,0

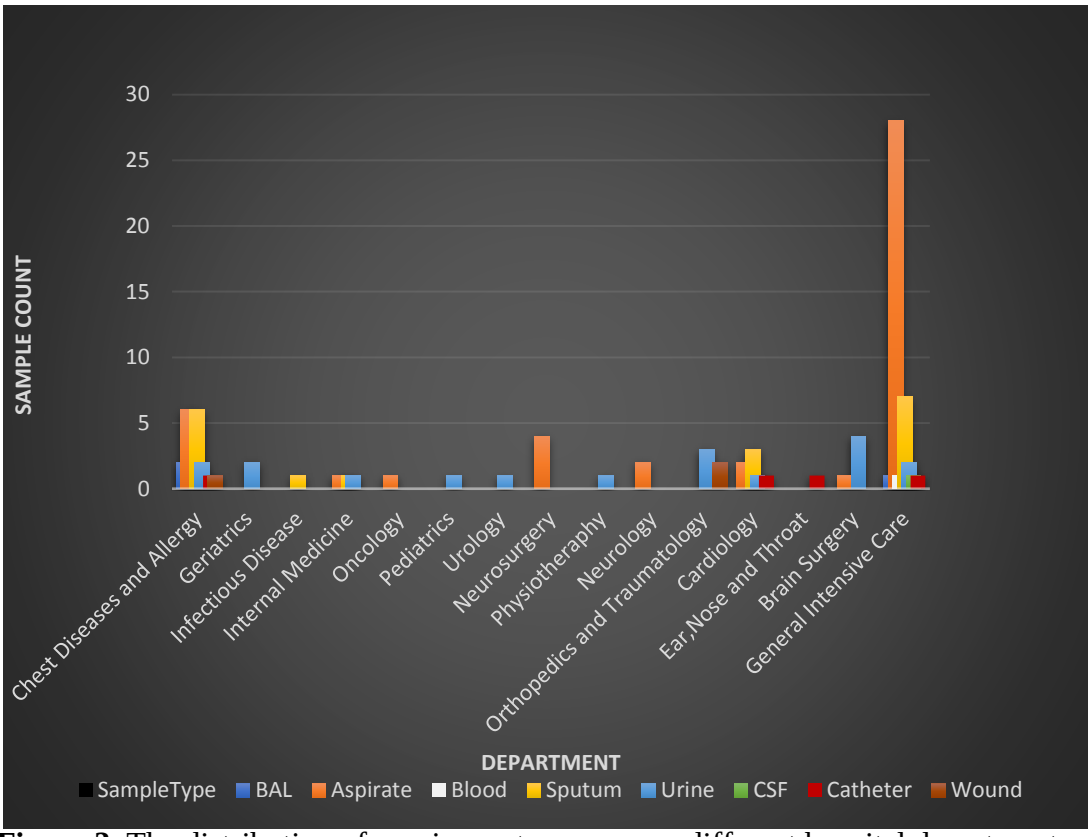


Figure 3. The distribution of specimens types among different hospital departments

4.2. Antimicrobial Susceptibility Testing Pattern

In this research, Antibiotic susceptibility tests were performed by full automated system Phoenix 100, the results as shown in Table 6 were described the sensitivity and the resistance of several important antibiotics as follow: Amikacin the sensitive were n=22 (23,7%) and the resistant were n=71 (76,3%), ciprofloxacin the sensitive were n=16 (17,2%) and the resistant were n=77 (82,8%), gentamicin the sensitive were n=21 (22,6%) and the resistant were n=72 (77,4%), imipenem the sensitive were n=17 (18,3%) and the resistant were n=76 (81,7%), levofloxacin the sensitive were n=17 (18,3%) and the resistant were n=76 (81,7%), meropenem the sensitive

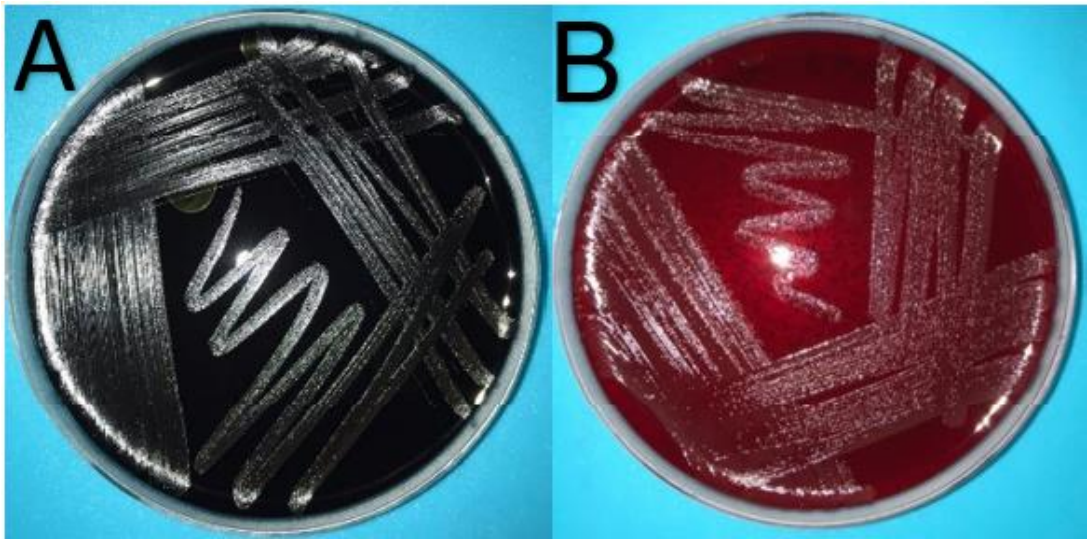
were n=16 (17,2%) and the resistant were n=77 (82,8%), trimethoprim/sulfamethoxazole the sensitive were n=29 (31,2%) and the resistant were n=64 (68,8%), and colistin the sensitive were n=87 (93,5%) and the resistant were n=6 (6,5%).

Table 6. Antimicrobial susceptibility pattern of Acb complex spp.

Antibiotic Name	Sensitive/Resistance	n (%)
Amikacin	Sensitive	22 (23,7%)
	Resistant	71 (76,3%)
Ciprofloxacin	Sensitive	16 (17,2%)
	Resistant	77 (82,8%)
Colistin	Sensitive	87 (93,5%)
	Resistant	6 (6,5%)
Gentamicin	Sensitive	21 (22,6%)
	Resistant	72 (77,4%)
Imipenem	Sensitive	17 (18,3%)
	Resistant	76 (81,7%)
Levofloxacin	Sensitive	17 (18,3%)
	Resistant	76 (81,7%)
Meropenem	Sensitive	16 (17,2%)
	Resistant	77 (82,8%)
Trimethoprim/Sulfamethoxazole	Sensitive	29 (31,2%)
	Resistant	64 (68,8%)

4.3. Biofilm Formation on CRA

Biofilms Formation were performed on Modified Congo Red Agar (MCRA), Table 7 shows the biofilm formation among *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex strains which were 87 (93,5%) out of 93 positive biofilm which appeared as black colonies on MCRA, and 6 (6,5%) out of 93 were negative which appeared red as shown in Picture 1, although the table shows the distribution of biofilm formation among various clinical specimens that were collected from.



Picture 1. Appearance of black colonies with a dry crystalline consistency which considered as strong evidence for ability to form biofilm (A), while the non-biofilm-producing strains form red colonies (B).

Table 7. Distribution of biofilm among various clinical samples

			Sample Type								Total
			BAL	Aspirate	Blood	Sputum	Urine	CSF	Catheter	Wound	
Biofilm	Negative	Count	0	2	0	4	0	0	0	0	6
		% of Total	0,0%	2,2%	0,0%	4,3%	0,0%	0,0%	0,0%	0,0%	6,5%
	Positive	Count	3	43	1	14	18	1	4	3	87
		% of Total	3,2%	46,2%	1,1%	15,1%	19,4%	1,1%	4,3%	3,2%	93,5%
Total		Count	3	45	1	18	18	1	4	3	93
		% of Total	3,2%	48,4%	1,1%	19,4%	19,4%	1,1%	4,3%	3,2%	100,0 %

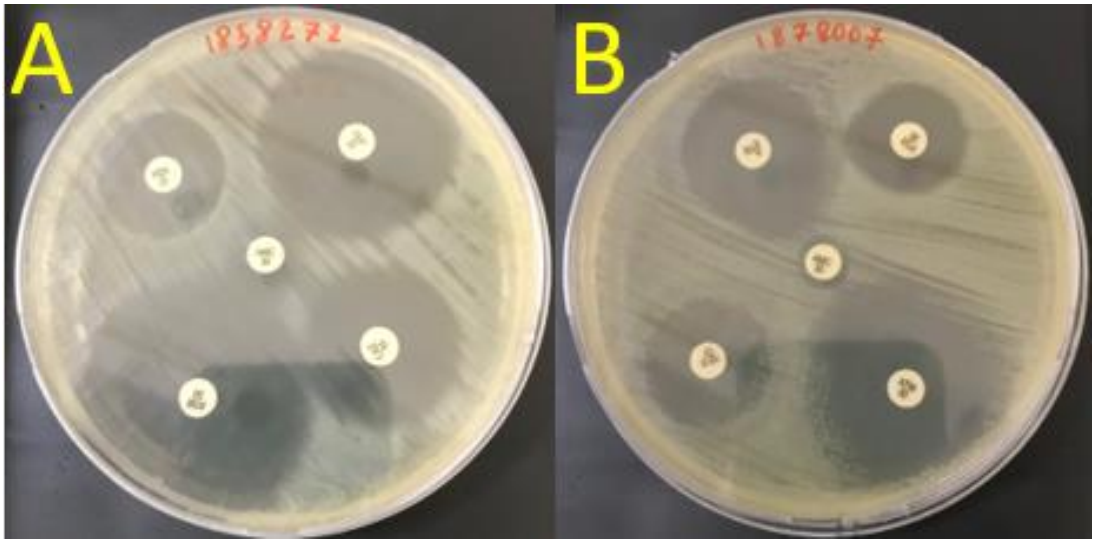
According to biofilm formation and antibiotic susceptibility test results, the majority of biofilm positive and antibiotic resistance were as followed: 86,2% (n=75) were biofilm positive among ciprofloxacin resistant, and 85,1% (n=74) were biofilm positive among levofloxacin and meropenem resistant, but in colistin 94,3% n=82 biofilm positive were sensitive and only 5,7% n=5 biofilm positive were resistance as shown in Table 8.

Table 8. Distribution of biofilm among antibiotics susceptibility test

Antibiotic name		Negative n (%)	Positive n (%)	Total	p
Amikacin	Sensitive	4 (66,7%)	18 (20,7%)	22	0,026
	Resistant	2 (33,3%)	69 (79,3%)	71	
Ciprofloxacin	Sensitive	4 (66,7%)	12 (13,8%)	16	0,007
	Resistant	2 (33,3%)	75 (86,2%)	77	
Colistin	Sensitive	5 (83,3%)	82 (94,3%)	87	0,338
	Resistant	1 (16,7%)	5 (5,7%)	6	
Gentamicin	Sensitive	4 (66,7%)	17 (19,5%)	21	0,022
	Resistant	2 (33,3%)	70 (80,5%)	72	
Imipenem	Sensitive	3 (50%)	14 (16,1%)	17	0,072
	Resistant	3 (50%)	73 (83,9%)	76	
Levofloxacin	Sensitive	4 (66,7%)	13 (14,9%)	17	0,010
	Resistant	2 (33,3%)	74 (85,1%)	76	
Meropenem	Sensitive	3 (50%)	13 (14,9%)	16	0,061
	Resistant	3 (50%)	74 (85,1%)	77	
Trimethoprim/ Sulfamethoxazole	Sensitive	4 (66,7%)	25 (28,7%)	29	0,064
	Resistant	2 (33,3%)	62 (71,3%)	64	
Total		6 (6,5%)	87 (93,5%)		

4.4. Detection of ESBL on DDST Method

DDST Disks containing cephalosporins (cefoxitin FOX 30 µg, ceftazidime CAZ 30 µg, cefepime FEP 30 µg) and (aztreonam ATM 30 µg) are placed to plates to a disk contain clavulanic acid (amoxicillin- clavulanic acid AMC 20/10 µg). In this study positive ESBL production was found 22,6% (n: 21) among all samples, and negative ESBL production was 77,4% (n: 72) as shown in Table 9 and Picture 2.



Picture 2 A positive ESBL is reported when the zones of inhibition around any of the cephalosporin disks are augmented in the direction of the clavulanic acid disc. The distance between the disks is important, and for cephalosporin 30 µg disks, 20 mm center to center was found to be ideal.

Table 9. Distribution of ESBL among various clinical samples

			Sample Type								Total
			BAL	Aspirate	Blood	Sputum	Urine	CSF	Catheter	Wound	
ESBL	Negative	Count	3	37	1	10	14	1	3	3	72
		% of Total	3,2%	39,8%	1,1%	10,8%	15,1%	1,1%	3,2%	3,2%	77,4%
	Positive	Count	0	8	0	8	4	0	1	0	21
		% of Total	0,0%	8,6%	0,0%	8,6%	4,3%	0,0%	1,1%	0,0%	22,6%
Total		Count	3	45	1	18	18	1	4	3	93
		% of Total	3,2%	48,4%	1,1%	19,4%	19,4%	1,1%	4,3%	3,2%	100%

According to ESBL production and antibiotic susceptibility test results, all of ESBL positive and negative among antibiotic sensitivity results were significant except colistin was not significant as shown in Table 10.

Table 10. Distribution of ESBL among antibiotics susceptibility test

Antibiotic name		ESBL			
		Negative n (%)	Positive n (%)	Total	p
Amikacin	Sensitive	7 (9,7%)	15 (71,4%)	22	0,000
	Resistant	65 (90,3%)	6 (28,6%)	71	
Ciprofloxacin	Sensitive	3 (4,2%)	13 (61,9%)	16	0,000
	Resistant	69 (95,8%)	8 (38,1%)	77	
Colistin	Sensitive	69 (95,8%)	18 (85,7%)	87	0,126
	Resistant	3 (4,2%)	3 (14,3%)	6	
Gentamicin	Sensitive	7 (9,7%)	14 (66,7%)	21	0,000
	Resistant	65 (90,3%)	7 (33,3%)	72	
Imipenem	Sensitive	3 (4,2%)	14 (66,7%)	17	0,000
	Resistant	69 (95,8%)	7 (33,3%)	76	
Levofloxacin	Sensitive	4 (5,6%)	13 (61,9%)	17	0,000
	Resistant	68 (94,4%)	8 (38,1%)	76	
Meropenem	Sensitive	3 (4,2%)	13 (61,9%)	16	0,000
	Resistant	69 (95,8%)	8 (38,1%)	77	
Trimethoprim /Sulfamethoxazole	Sensitive	13 (18,1%)	16 (76,2%)	29	0,000
	Resistant	59 (81,9%)	5 (23,8%)	64	
Total		72 (77,4%)	21 (22,6%)		

Further statistical was performed between biofilm formation and ESBL production, 18,3% (n: 17) were ESBL positive with biofilm positive, and 75,3% (n: 70) were ESBL negative with biofilm positive which were found significant as shown in Table 11.

Table 11. Distribution of ESBL among biofilm formation

			ESBL		Total	p
			Negative	Positive		
Biofilm2	Negative	n=	2	4	6	0,022
		% of Total	2,2%	4,3%	6,5%	
	Positive	n=	70	17	87	
		% of Total	75,3%	18,3%	93,5%	
Total		n=	72	21	93	
		% of Total	77,4%	22,6%	100,0%	

5. DISCUSSION

This study investigates the Antibiotic Resistance, ESBL, and Biofilm properties of *Acinetobacter* species strains that isolated from various clinical samples from Near East Hospital, according to the distribution of specimens in this study 93 *Acinetobacter* Acb complex specimens that collected from different site of the body, In this study, most of the isolates were obtained from respiratory specimens which were aspirate 48,4% (n=45), sputum 19,4% (n=18), and bronchoalveolar lavage 3,2% (n=3), which related to pneumonia infection. Also found that the majority of specimens were isolated from the general intensive care unit department with forty-one 44,1% and n=41.

Comparing to recent studies although the most isolates were obtained from respiratory specimens that were detected from Coskun et al. in 2019 in turkey noted that (tracheal aspirates were 54,2%, sputum were 12,5%, and bronchoalveolar lavage were 5,2%), Cicek et al. in 2014 in turkey noted that (tracheal aspirates were 17%, and sputum were 36%), and Alkasaby and El Sayed Zaki in 2017 in Egypt revealed that (Endotracheal secretion were 39,3% and sputum were 23,8%) (Alkasaby and El Sayed Zaki, 2017; Cicek et al., 2014; Coskun et al., 2019).

Gales et al in 2019 found that *Acinetobacter* Acb complex was taken among hospitalized patients which were 42,9% pneumonia from the aspirate sample in Europe. Although Ana C. Gales et al and Wong et al found that spp. of *Acinetobacter* Acb complex come from pandemic hospital-acquired infections, usually in intensive care units and/or immunocompromised patients. Although this pathogen can cause various types of infections, bloodstream infection and pneumonia were the most common infections according to the results of their study (Gales et al., 2019; Wong et al., 2017).

For the antibiotics resistance, recently *Acinetobacter* Acb complex was considered multi-drug resistance and/or extreme drug resistance all over the world

due to their highly resistance to different families of antibiotics. in comparison to the most recent researches of antibiotics resistance, this research shows 76,3% Amikacin resistant, 82,8% ciprofloxacin resistant, 77,4% gentamicin resistant, 81,7% Imipenem resistant, 81,7% levofloxacin resistant, 82,8% meropenem resistant, 68,8% trimethoprim/sulfamethoxazole resistant, and 6,5% colistin resistant.

The test results from Cicek et al. in 2014 in Turkey revealed: Amikacin resistant 63%, ciprofloxacin resistant 94%, gentamicin resistant 48%, imipenem resistant 95%, levofloxacin resistant 89%, meropenem resistant 94%, trimethoprim/sulfamethoxazole resistant 69%, and colistin resistant 0% (Cicek et al., 2014). The test results from Dahdouh et al. in 2017 in Spain revealed: Amikacin resistant 18,6%, ciprofloxacin resistant 89,8%, gentamicin resistant 54,2%, imipenem resistant 84,7%, levofloxacin resistant 88,1%, meropenem resistant 84,7%, trimethoprim/sulfamethoxazole resistant 88,1%, and colistin resistant 3,4% (Dahdouh et al., 2017).

The test results from Alkasaby and El Sayed Zaki in 2017 in Egypt revealed: Amikacin resistant 89,2%, ciprofloxacin resistant 92,9%, gentamicin resistant 92,9%, imipenem resistant 95,7%, meropenem resistant 95,7%, trimethoprim/sulfamethoxazole resistant 77,9%, and colistin resistant 3,2% (Alkasaby and El Sayed Zaki, 2017).

The test results from Chen et al. in 2018 in United States revealed: Amikacin resistant 78,1%, ciprofloxacin resistant 79,5%, gentamicin resistant 78,1%, imipenem resistant 75,3%, levofloxacin resistant 78,1%, meropenem resistant 75,3%, trimethoprim/sulfamethoxazole resistant 68,5%, and colistin resistant 2,7% (Chen et al., 2018).

The test results from Coskun et al. in 2019 in Turkey revealed: Amikacin resistant 72,9%, ciprofloxacin resistant 100%, gentamicin resistant 93,7%, imipenem resistant 100%, levofloxacin resistant 96,9%, meropenem resistant 100%, and colistin resistant 0% (Coskun et al., 2019).

The test results from Çağlan et al. in 2019 in Ankara, Turkey revealed: 53,0% amikacin resistant, 81,0% ciprofloxacin resistant, 60,5% gentamicin resistant, 81,5% meropenem resistant, and 4,2% colistin resistant (Çağlan et al., 2019).

The test results from de Freitas et al. in 2020 in Brazil revealed: Amikacin resistant 72,7%, ciprofloxacin resistant 100%, gentamicin resistant 81,8%, imipenem resistant 90,9%, and meropenem resistant 100% (de Freitas et al., 2020).

The test results from Metan et al. in 2020 in Ankara, Turkey revealed: 76,2% amikacin resistant, 100% ciprofloxacin resistant, 76,2% gentamicin resistant, 100% meropenem resistant, trimethoprim-sulfamethoxazole resistant 80,9%, and 57,1% colistin resistant (Metan et al., 2020)

Among the investigation of biofilm formation in *Acinetobacter* species, this study shows 93,5% (n=87) biofilm producers, and only 6,5% (n=6) non-biofilm producers. Comparing to recent studies that were detected from De Freitas et al. 2020 in Brazil biofilm formation among *Acinetobacter* species were 100%, Dahdouh et al. 2017 in the United States noted that 100% biofilm formation, Kumari et al. in 2020 in Eastern Nepal revealed that Biofilm was 77,7%, Sharma et al. 2019 in Nepal noted that biofilm was 9,3%, Dumaru et al. in 2019 in Dharan, Nepal noted that biofilm 54%, Neopane et al. in 2018 revealed that biofilm was 80% (Dahdouh et al., 2017; de Freitas et al., 2020; Dumaru et al., 2019; Kumari et al., 2020; Neopane et al., 2018; Sharma et al., 2019).

In the investigation of ESBL production among *Acinetobacter* species, this study shows 22,6% (n=21) positive ESBL, and 77,4% (n=72) negative ESBL. By reference to recent studies that were detected from Kumari et al. 2020 noted that ESBL positive were 5,98%, Sharma et al. in 2019 noted that ESBL positive were 31%, Dumaru et al. in 2019 revealed that ESBL positive were 15,87%, Alkasaby and El Sayed Zaki in 2017 noted that ESBL positive were 2,1%, Shamsuzzaman in 2017 noted that 9% were ESBL positive, and Coskun et al. in 2019 in turkey noted only 2% were positive to ESBL (Alkasaby and El Sayed Zaki, 2017; Coskun et al., 2019;

Dumaru et al., 2019; Kumari et al., 2020; Shamsuzzaman, 2017; Sharma et al., 2019).

In this study, the relationship among biofilm and antibiotic resistance was detected to be statistically significant for Amikacin, Ciprofloxacin, Gentamicin, and Levofloxacin. Dahdouh et al. in 2017, Neopane et al. in 2018, Dumaru et al. in 2019, and Kumari et al. in 2020, noted that a higher proportion of antibiotic resistance in biofilm formation in comparison to non- producers (Dahdouh et al., 2017; Dumaru et al., 2019; Kumari et al., 2020; Neopane et al., 2018).

In this study, the relationship among ESBL and antibiotic resistance was detected to be statistically significant for all antibiotics except Colistin. By reference to recent studies that were detected from Sharma et al. in 2019, Dumaru et al. in 2019, and Kumari et al. in 2020 noted that a higher proportion of antibiotic resistance in ESBL production in comparison to non-producers. Shamsuzzaman in 2017 noted that all the isolated ESBL producers showed resistance to all antibiotics but were sensitive to imipenem and colistin (Dumaru et al., 2019; Kumari et al., 2020; Shamsuzzaman, 2017; Sharma et al., 2019).

In this study, the association between positive biofilm formation and positive ESBL production was found 18,3% (n=17) which found that this proportion between biofilm formation and ESBL production statistically significant. By reference to recent studies that were detected from Dumaru et al. in 2019, Sharma et al. in 2019, and Kumari et al. in 2020 noted the association between positive biofilm formation and positive ESBL production was found 1,59%, 3,84%, and 1,2% respectively, which they revealed this proportion is not statistically significant (Dumaru et al., 2019; Kumari et al., 2020; Sharma et al., 2019). However, owing to various circumstances that may be due to variations in location, specimen structure in the sample groups, condition, and antibiotic usage, a few changeable findings are seen in some reports.

6. CONCLUSION

The results from this study indicate that *Acinetobacter* species are not sensitive to several antimicrobial agents available that make this nosocomial pathogen one of the most important microbial challenges to be managed in the future. The conspicuous prevalence of biofilm-producing and multidrug-resistant bacteria in our institutions presents an impression of potential challenges in our region of the world. Routine observation of biofilm formation and beta-lactamases; thus, along with stringent enforcement of infection management and prevention practices may be advised in clinical laboratories.

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6. CURRICULUM VITAE

Name	Mohammad	**Surname**	Malkawi
Place of Birth	Irbid, Jordan	**Date of Birth**	7-10-1996
Nationality	Jordanian	**Tel**	+905391037748 +962797594543 +962777859474
E-mail	Mohammadmalkawi380@gmail.com		

Educational Level

	Name of the Institution where was graduated	**Graduation year**
Postgraduate/Specialization	Near East University, Northern Cyprus.	2020
Masters	Near East University, Northern Cyprus.	2020
Undergraduate	Jordan University of Science & Technology (JUST), Irbid-Jordan.	2018
High school	Al-Waleed bin Abdul Malik School, Irbid-Jordan.	2014

Masters Thesis	
Title:	Investigation of Antibiotic Resistance, Esbl, and Biofilm Properties of Acinetobacter Species Strains Isolated From Various Clinical Samples
Supervisor:	Assoc. Prof. Dr. Meryem GÜVENIR

Job Experience

Duty	Institution	Duration (Year - Year)
Medical Laboratory technician	Prince Rashed Bin Al Hasan Military Hospital, Irbid-Jordan	Feb 2018 – May 2018
Medical Laboratory technician	Ibn Al-Nafis hospital, Irbid-Jordan	For three months

Courses and Certificate

Name	Name of the Institution where take place	year
Medical Lab Technician Certificate	The Ministry of Health, Jordan.	2018
Medical Lab Technician Trainee Certificate	King Hussein Medical Center, Jordan	2018
Mathematical Modeling in Health Certificate (Attendance)	DESAM Institute, Near East University, Nicosia	2019
Parasitology Academic Course: Essential and Application	Turkish Microbiology Society, TMC-KKTC Microbiology Platform, Nicosia	2020

Foreign Languages	Reading comprehension	Speaking*	Writing*
English	Excellent	Excellent	Excellent

Foreign Language Examination Grade

YDS	ÜDS	IELTS	TOEFL IBT	TOEFL PBT	TOEFL CBT	FCE	CAE	CPE
•			•	•				

Computer Knowledge

Program	Use proficiency
SPSS	Excellent
Python Programming Language	Excellent
Common Computer Programs and Skills	Excellent