Ministry of Higher

Education and Scientific

Research

University of Babylon

College of Medicine

Genotypic Diversity and Characterization of CRISPR-Cas Systems in Multidrug-Resistant *Acinetobacter baumannii* Isolates

A Thesis

Submitted to the Council of College of Medicine-University of Babylon in Partial Fulfillment of the Requirements for the Degree of Master in Medical Microbiology

By

Nadia Abid Al Abass Bayram

B.Sc.in Microbiology\College of Science University of Babylon 2001

Supervised by

Professor

Dr. Maysa S. AL-Shukri

2022 A.D **1443 A.H**

بسم الله الرحمن الرحيم

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ
أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا
تَعْمَلُونَ خَبِيرٌ

صدق الله العظيم

Decision of Examination Committee

We certify that we have read this thesis entitled : (**Genotypic Diversity and Characterization of CRISPR-Cas Systems in Multidrug-Resistant *Acinetobacter baumannii* Isolates)** and as an examining committee, examined the student "**Nadia Abid Al Abass Bayram**" in its content and in our opinion, it meets standard of thesis for the degree of Master in Medical Microbiology with (Excellent) estimation.

Professor
Dr. Ahlam Kadhum Naeem
Faculty of Education for Girls
University of Kufa
(Chairman)

Assist. Prof.
Dr. Zainab Adil Ghani Chabuck
College of Medicine
University of Babylon
(Member)

Professor
Aalaa fahim Abbas
College of Science
Al-Qadisiyah University
(Member)

Professor
Dr. Maysa S.AL-Shukri
College of Medicine
University of Babylon
(Member/ supervisor)

Approved by the College Committee on Post-graduate Studies.

Prof. Dr.
Mohanned Abbass Nori Al-Shalah
Dean of College of Medicine
University of Babylon

Certification

We certify that this thesis entitled: (**Genotypic Diversity and Characterization of CRISPR-Cas Systems in Multidrug-Resistant *Acinetobacter baumannii* Isolates)** was prepared under our supervision by "Nadia Abid Al Abass Bayram" at the Department of Microbiology- College of Medicine/ University of Babylon, as a partial fulfillment for the requirements for the degree of Master of Science in Medical Microbiology and this work has never been published anywhere.

Professor
Dr. Maysaa S. AL-Shukri
University of Babylon
College of Medicine

/ / 2022

In view of the available recommendation, I put forward this thesis for debate by the examining committee

Professor
Dr. Hayam khalis Al-Masoudi
Head of Microbiology Department University of Babylon
College of Medicine

/ / 2022

Dedication

To soul of my mother … the shade that I port to at every time

To all my family, who helped, supported and surrounds me with love…

To those who have innocent smiles, clear souls, diamond hearts who are kind and helpful .

I dedicated this humble work …

Nadia

Acknowledgment

Firstly, I would like to thank *Allah* for giving me the bravery, and power to complete this research.

I would like to express my gratitude to my supervisor **Prof. Dr. Maysa S. Al-Shukri** for her efforts to advice, guidance and communion to accomplish my work. Also, I would like to express my gratitude to **Asst. Prof. Dr. Rana A. Ghaleb** which has never hesitated to assist with nanoparticle scale work in research.

Special thanks to **Assist. Lec. Duha Muhanad Bayram** for helping me to get EndNote software tool for managing bibliographies and citations. Grateful thanks to all patients involved in this study. **WITH H**onest thanks to the staff of the Department of Microbiology at the College of Medicine, University of Babylon for their efforts to communicate the information's during our study. Finally, I would like to thank staff of Laboratory department MERJAN Teaching Hospital for to help me during my work.

Nadia

Summary

Acinetobacter baumannii is a Gram-negative, opportunistic pathogen, causing severe infections difficult to treat. Total of (150) clinical specimens were collected from patients suffering from different infections.

To detect the CRISPR /Cas system in *A.baumannii* and genotyping methods for *A.baumannii* isolates by ERIC and BOX

All clinical specimens were cultured on blood agar, MacConkey agar and CHROMagar then identification of *A. baumannii* was confirmed by biochemical test and vitek 2 system.

The antibiotic sensitivity was carried out using a standerd Kirby - Bauer method for ten different antimicrobials against *A. baumannii*. Followed by biofilm detection by tissue culture plate method.

Moreover, $AgTIO_2$ and SWCN-OH effect were examined against *A. baumannii* isolates growth and biofilm separately by making five double serial dilutions at (1/2, 1/4, 1/8, 1/16 and 1/32) form stock solutions (concentration 1000 μg/ml).

To access the presence of *cas* gene, DNA were firstly extracted from all *A. baumannii* isolates and multiplex PCR was done to amplify six different *cas* genes.

For genetic diversity BOX-PCR and ERIC-PCR were done and the obtain result analyzed using PyElph version 1.4. The dendrograms were constructed using an unweighted pair group method with arithmetic mean (UPGMA).

The results indicated that *A. baumannii* was responsible for 23/150 (15.3percent) of the samples, 12/45 isolates (26.6percent) were identified from sputum, 5/35 isolates (14.2percent) from burns, and 3/30 isolate (10percent) from urine, 3/40 isolates (7.5percent) from wounds, And all of these isolates appear to be resistance for Cefepime (100%) while in case of other antimicrobial agent the following result appeared, (91%) to

Trimethoprim-Sulfamethoxazole, (80%) to Gentamicin, Amikacin, (77%) to Ciproloxacin, Ceftazidime, Levofloxacin, (73%) to Amoxicillin-clavulanica acid, and (70%) to Meropenem, , while low resistance percentage (50%) to Tetracyclin.

In this study all *A. baumannii* isolates able to produce strong biofilm formation capacity. So, the effect of $AgTIO_2$and single walled carbon nanotubes (SWCN-OH) were tested upon its growth and biofilm and the results showed that there was a highly significant decrease in growth and biofilm of bacteria after exposure to the double serial dilutions of $AgTIO_2$ and single walled carbon nanotubes nanoparticles, with more effective by SWCN-OH.

Genetic analyses result which aim to detect *cas* genes of *A. baumannii* were appear that all studied samples contain this gene as following percentage, for *Cas1* (506 bp), *cas*2 (196 bp), and *cas3* (850 bp) gene amplicons were found in 5(25%), 3(15%), and 9(45%) of the isolates respectively. The *cas* 5 gene was discovered in 3(15%) of isolates, the *cas* 6 gene in 7(35%) of isolates, and finally the *cas* 9 gene in 6(30%) of isolates.

In case of I–F CRISPR–*Cas* (Which is belongs to the Type I system) this gene (~157bp) was identified in 7 out of 20 in different sample types including: 3(100%) urine, 2(22.22%) sputum, 1(20%) burn and 1(33.3%) wound.

BOX and ERIC-PCR for the detection of phylogenetic diversity of *A. baumannii* isolated from different samples, in general, 20 isolates characterized using the two molecular techniques had comparable number of bands with some degree of polymorphism. The two molecular techniques generated 2 main clusters and the results of dendrogram of these techniques reveals that 20 polymorphic variants between *A. baumannii* clinical isolates detect by ERIC and BOX PCR.

In conclusion, our data exhibits an association between virulence determinants and biofilm formation among Iraqi MDR *A. baumannii* strains and these data help to understand bacterial resistance and establishing in Iraqi hospitals. In addition to effectiveness of nanoparticles that may help in future to control spreading after other enrichment experimental supplementary studies.

Table of Contents

List of tables

List of figures

List of Abbreviation Expansion

Abbreviation	Expansion
AFLP	Amplified fragment length polymorphism
Ag-TiO2	Silver-doped titanium dioxide
AHL	Acyl homoserine lactones
AMEs	Aminoglycoside modifying enzymes
ARDRA	Amplified ribosomal DNA restriction analysis
Bap	Biofilm-associated protein (Bap)
BSA	Bovine serum albumin
CAP	Community acquired pneumonia
cas	CRISPR-associated protein
CFU	Colony forming unit
CLSI	Clinical and Laboratory Standards Institute
CRAB	Carbapenem-resistant *A. Baumannii*
CRISPR/Cas	Clustered regularly interspaced short palindromic repeats
D.W	Distilled water
DDT	Disc diffusion method (DDT)
DNA	Deoxyribonucleic acid
EPS	Extracellular polymeric substances
ERIC	Enterobacterial repetitive intergenic consensus
GNB	Gram-negative bacteria
HAIs	Hospital-acquired infections
ICUs	Intensive care units
LOS	Lipoolgiosaccharide
LPS	Lipopolysaccharide
MBL	Metallo-β-lactamases
MDR	Multidrug drug-resistant
MGEs	Mobile genetic elements
MLST	Multilocus sequence typing
NPs	Nanoparticles
OD	Optical density (OD)
Omps	Outer membrane proteins

OMVs	Outer membrane vesicles
OXA	Oxacillinases
PAI	Pathogenicity islands
PCR	Polymerase chain reaction
PDR	Pandrug drug-resistant
PFGE	Pulsed-field gel electrophoresis
QS	Quorum sensing
RAPD	Rapid amplification of polymorphic DNA
RND	Resistance nodulation cell division
ROS	Reactive oxygen species
STs	Sequence types
SWCNT-OH	Single walled carbon nanotube
TBE	Tris-Borate-EDTA buffer
TCP	Tissue culture plate method
TE	Tris-EDTA buffer
UK	United kingdom
USA	*United States of America*
UTIs	Urinary tract infection
VAP	Ventilator-associated pneumonia
WHO	World health organization
XDR	Extensively drug-resistant

Chapter One

Introduction and Literatures Review

1.1 Introduction

Acinetobacter baumannii is an opportunistic Gram-negative bacterium considered one of the most prevalent pathogens associated to nosocomial infections, especially among immunocompromised patients. Its ability to survive in hostile conditions, its high level of intrinsic and acquired antimicrobial resistance and the ease with which it spreads within and between health care units worldwide has made *A. baumannii* a successful pathogen in hospital settings(Hamidian and Nigro, 2019).

A. baumannii is primarily associated with wound and burn infections and ventilator associated pneumonia but is also an important cause of urinary tract infections and nosocomial bacteremia (Ramirez *et al.*, 2020). infections caused by this organism are often found in intensive care units (ICUs) in patients with immunosuppression, in elderly patients with underlying illnesses such as malignancy and burns, in patients undergoing aggressive therapeutic procedures and in those who use broad-spectrum antibiotics (Falah *et al.*, 2019) .

A. baumannii exhibits intrinsic resistance to many classes of antimicrobial agents and is further capable of developing resistance to virtually all other classes of agents used in the clinical practices to treat Gram-negative infections(Lee *et al.*, 2017).

The rapid increase in the clinical relevance of *A. baumannii* was precipitated by hard to treat and increasingly antibiotic-resistant lineages that emerged from an ancestral susceptible pool (Diancourt *et al.*, 2010).

A key component in the success of *A. baumannii* has been its remarkable ability to develop antibiotic resistance through mutation (Yoon *et al.*, 2013), gene amplification (McGann *et al.*, 2014), or horizontal acquisition of resistance genes (Doi *et al.*, 2015). Of particular concern is the emergence of carbapenem-resistant *A. baumannii* (CRAB), resulting in

infections treatable only with “last-line” antibiotics (Butler *et al.*, 2019). For example, although its nephrotoxicity, colistin (polymyxin E) is considered the last-line drug for treating infections caused by CRAB, but colistin-resistant strain has been isolated (Oikonomou *et al.*, 2015). Although many factors have contributed to this predicament, the absence of new antibiotics can be directly traced to the near-complete withdrawal of pharmaceutical companies from antibiotic development in the late 1980s (Conly and Johnston, 2005).

The formation of biofilm contributes to *A. baumannii* adhere to various biological and non-biological surfaces, such as vascular catheters, cerebrospinal fluid shunts, and other medical devices. Biofilm is defining composed of extracellular polymeric substances (EPS) that surrounding microorganisms, which mediates microbial adhesion and makes a lifestyle that is totally different from the planktonic state. Indeed, biofilm formation presents a significantly higher antimicrobial resistance than planktonic cells, resulting in harder eradication and easier recurrence in biofilm-associated infections. Biofilm formation is a complex process and regulated by a variety of factors. Taking *A. baumannii* for example, biofilm associated protein (Bap) is involved in the initial adhering of biofilm, promoting the maturation of biofilm and maintaining the structure of mature biofilm (Li *et al.*, 2021).

Different typing systems are based on phenotypic tests and molecular techniques. Biotyping, serotyping and bacteriocin typing are examples of phenotypic typing methods most of which have now been replaced by molecular typing systems such as plasmid profile analysis, ribotyping, pulsed-field gel electrophoresis (PFGE) and enterobacterial repetitive intragenic consensus-polymerase chain reaction (ERIC-PCR)(Falah *et al.*, 2019).

In *A. baumanni*, repetitive sequences of the gene which are called symmetric elements are often seen in the non-coding fragment of the DNA. Regarding the variable number and length of these repeat sequences, some primers have been designed. The length and number of bands obtained for each isolate are variable and the strains could be grouped according to the diversity of the bands(Falah *et al.*, 2019).

Genotyping allows investigation of clonal spread and can be used to identify the source of the original infection**.**

1.2 Aim of this study

To detect the CRISPR /Cas system in *A.baumannii* and genotyping diversity for *A.baumannii* isolates by ERIC and BOX this were done by

1. Isolation and identification of *A.baumannii* from different clinical specimen and identified by traditional methods and conferes by vitek2 system.
2. Antibiotic sensitivity test profile of *A.baumannii* .
3. Detection of biofilm production .
4. Study the effect of Ag-Tio2 and SWCN-OH nanoparticles on bacterial growth and biofilm formation.
5. Detection different types of *cas* gene by PCR methods.
6. Genotyping methods by ERIC and BOX for all isolates of *A.baumannii* .

1.3.1 Characterization of *A. baumannii*

The most common properties that characterize genus *Acinetobacter* are: according to gram staining these bacteria gram negative appeared in short rod with 1.5μm in diameter and 1.5-2.5μm in length during the logarithmic phase and coccobacilli or coccoid during the stationary phase and according to cultural properties this genus considered strict aerobic with non-motile also no-fermenting and fastidious colonies, additionally positive to catalase and negative to oxidase (Monem *et al.*, 2020).

On culture media most of the clinical isolates of genus *Acinetobacter* grow at 35 - 37°C or higher (especially species *baumanni*) while others grow only at lower temperatures, appearance of colonies on blood agar is creamy or white and mucoid or smooth colonies but on MacConkey agar colonies-appearance is faint pink color which indicates the non-lactose fermenter isolates (Biswas and Rather, 2019), on CHROMagar Acinetobacter which is selective medium developed for the rapid identification of *A. baumannii* with the appearance of colonies as convex and Light purple with halo with rounded colonies. This medium contains incorporates enzymatic substrates that enable color based preliminary identification of bacterial colonies recovered within 18 to 24 h of incubation (Perry, 2017).

1.3.2 Epidemiology *A. baumannii*

A. baumannii is almost be considered new pathogen and as many researchers suggested the first appearance of this pathogen was in Iraq specifically in military Iraqi War and was called “Iraqibacter”, infections of this pathogen associated with military and injured soldiers in combat due to direct environmental contamination of wounds due to considering *A. baumannii* to become the potential and serious problem in military hospital (Dewachi, 2019).

The occurrence of *A. baumannii* in different habitats like soil, water, wound and infections even in arthropods and food products make them one of the most epidemic pathogens related to their ability to cause diseases like septicemia and wound sepsis also bacteremia as well as meningitis, pneumonia linked with the ventilator and the respiratory and digestive tracts of humans, the risk of these pathogens related to their resistance-raising to many drugs and increase risks to human (Almasaudi, 2018).

The outbreaks of *Acinetobacter* are seen in critical care and burn care units and involved in mechanical ventilation cases in addition to many cases with Nosocomial *Acinetobacter* infections were identified outside Intensive Care Units (ICUs). Most of such cases being infected through the respiratory tract however a few have unidentified primary site bacteremia. The most of *A. baumannii* isolates commonly return to a sole clone, which have distributed in geographically distinguished regions and in specified institutional outbreaks in European (Zowawi *et al.*, 2015).

A. baumannii is resistant to dehydration also ultra-violet radiation in addition to common chemical sanitizers and detergents making it extremely difficult to eradicate and the increasing resistance of *A. baumannii* to antimicrobial drugs, including carbapenems, and resistance to dehydration and disinfectants contribute to its persistence in hospital environments and propensity to cause outbreaks (Amudhan *et al.*, 2011).

1.3.3 Pathogenicity of *A. baumannii*

Pathogenicity , meaning the ability of a microorganism to cause disease, in the past, members of the genus *Acinetobacter* ,is considered global organisms and is responsible for causing opportunistic infections, they are considered to be low grade pathogens, number of reports about community acquired pneumonia cases have referred to the high grade of

pathogenicity back to the members of this genus which led them to cause invasive diseases(Bergogne-Bérézin *et al.*, 2020).

The persisting encourages the clonal of bacteria to spread simply from the person-to-person and contaminate environment, the three major factors responsible for the strength of this bacterium are its resistance to major of antimicrobial agents, desiccation and disinfectants (Meumann *et al.*, 2019).

In human specimens the *Acinetobacters* were the most commonly isolated non-fermenters after *Pseudomonas aeruginosa*. Although 1-3% of health care-associated infections are caused by *Acientobacter* spp. but these bacteria have a little risk to healthy people. However, people with weakened immune systems, chronic lung disease and diabetes may be more susceptible to be infected with these bacteria (Almasaudi, 2018). The infection process is usually associated with various risk factors especially in ICUs these factors include the use of contaminated equipment such as ventricular or urinary catheters as well as the previously colonized health care staffs in addition to prolonged staying at intensive care units increased the risk of colonization and infection. Colonization is not necessarily symptomatic but it definitely increases the likelihood of causing subsequent infections which will proceed if the host natural barriers were weakened by trauma, surgery or other invasive procedures (Meschiari *et al.*, 2021).

1.3.4 Infections by *A. baumannii*

A. baumanni has ability to survive in the high stress environments as well as resistance to many classes of antibiotics which make them for surviving and spreading as a nosocomial bacterium specially in patients due to increase in morbidity and mortality(Monem *et al.*, 2020). There is a massive type of infections detected including

respiratory infections, bloodstream infections, skin and soft tissue infections and many other types.

1.3.4.1 Respiratory Infections

Ventilator-associated pneumonia (VAP) disease that is associated with MDR *A. baumannii*-infections and considered the most mortal disease in ill patients, according to statistical researches 8 to 14 percent of VAP, caused by *A. baumannii* occurred in the United States and Europe. But in Asia it occurred in rates higher than 19 to more than 50 percent. Since the VAP caused and induced by *A. baumannii* are considered dangerous to patients, community acquired pneumonia (CAP) which caused by this pathogen has been increased and concerned disease. This disease characterized by high rates of bacteremia as well as high mortality rate especially in tropical regions where it specially affects the alcoholic individuals , diabetes mellitus as well as smokers and patients with chronic lung disease (Jaruratanasirikul *et al.*, 2019).

1.3.4.2 Blood stream Infections

Bloodstream infections because of contaminated catheters (intravenous) are caused by *A. baumannii*, especially those in health care units or catheters of respiratory tract still be the big source in threat life, and increase the mortality rate reaching to 40%, on the other hand 13 % of blood stream infections occurred in patients specifically in those who are undergoing neurosurgery are caused by *A. baumannii*. 90 % of these pathogens were carbapenem resistant (Tsitsopoulos *et al.*, 2016).

1.3.4.3 Skin and Soft Tissue Infections

The isolation of *A. baumannii* from skin and soft tissue that occurs in repeated times from patients with severe burns as well as wounds even in trauma especially from soldiers who injured during wars, among them

patients with severe burns were in a tertiary care burn center infected by *A. baumannii* also the comorbidities by long stay cause high mortality for those patients compared to patients without infection by *A. baumannii*. Therefore *A. baumannii* pathogens which infected wounds (especially in wars) represent more dangerous and threat life. So that these pathogens should be prevented by treating the non-healing wounds which are possibly develop to biofilms with strong vital antimicrobial resistance medications (Dallo and Weitao, 2010).

1.3.4.4 Urinary Tract Infections

A. baumannii isolates are the occasionally causes of urinary tract infection; these isolates are responsible for 1.6 percent of intensive care units acquired urinary tract infection; it is commonly attached to catheter-related infection. It is uncommon for this bacteria to lead to complexed urinary tract infection in outside of hospital depending on results of study by (Di Venanzio *et al.*, 2019).

1.3.4.5 Meningitis

Meningitis disease caused by *A. baumannii* still increasing especially in intensive care neurosurgery units and threats patient life with mortality reaching to 70 %, especially in patients on cerebrospinal fistulae in addition to patients who are receiving therapy after surgery(Sari *et al.*, 2021).

In 2019 large number of disease states of meningitis in post neurosurgical patients which caused by *A. baumannii* show that 21% of isolates were extensively drug-resistant XDR isolates which have sensitivity to only colistin and tigecycline. On the other hand, the pediatric meningitis cases with high mortality rate specifically in China are caused by the same pathogens cultures of cerebrospinal fluid showed the multidrug

drug-resistant MDR also XDR as well as Pandrug drug-resistant PDR *A. baumannii* after the neuro surgery (Xiao *et al.*, 2019).

1.3.5 Virulence factor of *A. baumannii*

Virulence factor are molecules expressed and separated by pathogens (bacteria, viruses, fungi, and protozoa) they help the microorganisms overcome host defenses and colonize or invade host cells, this includes.

1.3.5.1 Outer membrane protein A

Outer membrane proteins (Omps) of Gram-negative bacteria are known to be key players in bacterial adaptation and pathogenesis in host cells. Porins play a variety of roles depending on the bacterial species, including the maintenance of cellular structural integrity, bacterial conjugation and bacteriophage binding, antimicrobial resistance and pore formation to permit the penetration of small molecules (Iyer *et al.*, 2017).

Outer membrane protein A (OmpA) consider the first *A. baumannii* virulence factor that described and confirmed in vivo. OmpA is a main porin protein abundant in the outer membrane of *A. baumannii* that has an essential role in pathogenesis and keeps the structure of the outer membrane, and it is found in all strains (Hood *et al.*, 2010).

OmpA, also has another significant function, that consists of adhesion to host epithelial cell, target mitochondria that leads to cause mitochondrial dysfunction by activation of caspase and cytochrome C , also it passes to the nucleus and leads to cleavage DNA (Smani *et al.*, 2014).

1.3.5.2Outer membrane structures

Generally, Gram-negative bacteria possess lipopolysaccharide (LPS), which is an essential component in cell envelope and complicated in virulence. In *A. baumannii*, LPS molecules comprises of an O-

polysaccharide chain (O-antigen), a glycosylation system, which participates in the synthesis of the O-antigen , and was recognized to be important in biofilm formation (Li *et al.*, 2020).

Lipid A, is the other part of LPS, also play a significant role in septic shock, LPS makes the cell latent targets for antibacterial drug advance, and it is involved in serum resistance, and the inflammatory response in systematic infections (Antunes *et al.*, 2014).

The damage of LPS leads to weakening the virulence factor, antibiotic sensitivity, and reduction in vitro growth rates (Bojkovic *et al.*, 2015).

Furthermore, several studies demonstrate that colistin resistance is associated with the loss of LPS production. The additional constituent of the outer membrane of *A. baumannii* is capsular polysaccharides; these polysaccharides are composed of repeating carbohydrate units and their purpose as glycan shields that cover the whole bacterium and keep it from external pressures, and it is involved in the resistance to desiccation, because of the capacity of the capsule in *A. baumannii* to maintain water and grow in a biofilm under dry conditions (Martín-Aspas *et al.*, 2018).

1.3.5.3 Outer membrane vesicles (OMVs)

Another important virulence factor of *A. baumannii* is outer membrane vesicles (OMVs), secreted by several Gram-negative bacteria. Outer membrane vesicles are suggested as vehicles for secretion of protein discrete from type I to VI secretion system. These spherical bilayered OMVs ranged from 20 to 200 nm in diameter and emitted from the outer membrane (Li *et al.*, 2015).

These vesicles hold outer membrane proteins, proteolytically unstable enzymes, periplasmic proteins, and other nonprotein molecule such as lipids, DNA and RNA. They possess essential role in intercellular and intracellular signaling, transfer of virulence factors, cytotoxicity and immune response induction. *A. baumannii* OMVs exhibited hemolytic and leukotoxic actions and phospholipase C (Jha *et al.*, 2017).

1.3.6. Multidrug Resistance of *A. baumannii*

Multidrug-resistant pathogens posses serious threats in healthcare settings worldwide. For the past number of years, antimicrobial discovery and resistance development to new antimicrobials occurred almost at the same time. Not surprisingly, *A. baumannii*, similarly to other bacteria, also acquired resistance to newly developed antimicrobial agents (Clark *et al.*, 2016).

Various patterns of resistance found 22 antimicrobial agents belong to nine categories: aminoglycosides, carbapenems, fluoroquinolones, penicillins + β lactamase inhibitors, extended spectrum cephalosporins, folate pathway inhibitors, polymixins, and tetracyclines (Kanj and Kanafani, 2011).

A. baumannii can become resistant to a variety of antibiotics via intrinsic and acquirement mechanisms. Its ability to acquire drug resistance genes from other human pathogens is not well understood. However, considering the capability of the *A. baumannii* genome to exchange genetic material both within and between species, it is quite likely that these bacteria may have evolved toward enhanced pathogenicity (Lupo *et al.*, 2018).

Severe hospital-acquired infections caused by *A. baumannii* involve the use of carbapenems as highly effective drugs of choice used for the

treatment of such infections. Because of their broad spectrum, carbapenems are often active against microorganisms resistant to other antimicrobial compounds, and they are frequently used to treat complicated bacterial infections (Papp-Wallace *et al.*, 2011).

Over the last few years, *A. baumannii* MDR strains became increasingly resistant to carbapenems, the drug of choice to treat severe infections caused by these bacteria. The main cause of carbapenem resistance is the presence of oxacillinases (OXA), which belong to class D β-lactamases. Over 400 OXA enzymes encoded by chromosome- or plasmid-located genes were characterized. Other classes of β-lactamases: class A, class B (metallo-β-lactamases, MBL), and class C (AmpC) were also identified in *A. baumannii* strains (Vrancianu *et al.*, 2020).

Another mechanism of *A. baumannii* resistance is associated with enzymatic modification of the antimicrobial molecule. One of the best examples of resistance via modification of the drug is the presence of a large group of aminoglycoside-modifying enzymes (AMEs). These enzymes possess unique substrate specificity and modify amino- or hydroxyl- groups of the aminoglycosides. There are three different types of AMEs, acetyltransferases, nucleotidyl transferases, and phosphotransferases, while all of them were identified in *A. baumannii* isolates (Vrancianu *et al.*, 2020).

Proteomic analysis of *A. baumannii* MDR strains shows protein variability that could be correlated with the appearance of resistance phenotypes, especially OMPs, which are involved in cellular drug uptake or efflux. The emergence of an antibiotic resistance level is often related to diverse variations in the expression of OMPs (Eze *et al.*, 2018).

Another common mechanism of antibiotic resistance in *A. baumannii* is alteration of the target site or cellular functions (Fernández

and Hancock, 2012). This often results from spontaneous mutation of a bacterial gene on the chromosome. Modification of the target site results in decreased affinity for the drug molecule. In an alternative pathway, bacteria produce new proteins that protect the target against an antibiotic. Examples of drugs affected by this mechanism include fluoroquinolones and tetracyclines (Singh *et al.*, 2013).

In *A. baumannii*, point mutations in the *gyrA/parC* topoisomerases result in fluoroquinolones resistance, whereas a mutation in *rpsJ*, the gene that encodes the ribosomal S10 protein, is responsible for tigecycline resistance (Beabout *et al.*, 2015).

Another interesting example is the mechanism responsible for colistin resistance. Positively charged colistin kills bacteria by interacting with the negatively charged lipid A and destabilization of the OM. Colistin initially binds to the lipid A moiety of lipopolysaccharide (LPS), resulting in destabilization of the Gram-negative outer membrane , Alarmingly, as the use of colistin has increased, so have reports of colistin-resistant *A. baumannii* strains , Previously, we have shown that *A. baumannii* can become resistant to colistin via mutations in the lipid A biosynthesis genes *lpxA, lpxC,* and *lpxD,* leading to the complete absence of LPS from the outer membrane , significantly, LPS loss has been observed in clinical isolates(Kamoshida *et al.*, 2020) .

Among several types of efflux pumps that confer multidrug resistance, the resistance nodulation cell division (RND) efflux systems (AdeABC, AdeFGH, AdeIJK) are the most prevalent in *A. baumannii*. The AdeABC pump, found in 80% of *A. baumannii* isolates, extrudes a wide range of antibiotics, including β-lactams, aminoglicosides, fluoroquinolones, tetracyclines-tigecycline, macrolides–lincosamides, and chloramphenicol (Abdi *et al.*, 2020)).

1.3.7 General Introduction to Biofilms in *A. baumannii*

Biofilms are multicellular groups of single or multiple bacterial species enclosed in extracellular polymeric substances (EPS) which comprise polysaccharides, proteins, and nucleic acids secreted by bacteria. The structure of mature biofilms is often very complex with clusters of bacterial cells separated by fluid-filled channels. Diffusion of nutrients and oxygen is limited in biofilms; therefore, the environmental conditions are not homogeneous throughout a biofilm. This leads to the formation of heterogeneous cell subpopulations adapted to local microenvironments. Biofilm-dwelling bacteria are more resistant to antibiotics and other stressors than planktonic cells (Monem *et al.*, 2020).

Numerous studies have demonstrated that *A. baumannii* can form a biofilm on abiotic surfaces, such as glass or plastic (Espinal *et al.*, 2012). This is an important phenotype, as it is believed that biofilm formation facilitates the transmission of pathogens, and provides a selective advantage by allowing organisms to survive under less than favorable environmental conditions (Hall-Stoodley and Stoodley, 2005).

The ability of bacterial pathogens to form biofilms on various surfaces such as mucosal membranes and medical indwelling devices allows them to persist despite antibiotic therapy and the host immune defense. As a result of the ability to form a biofilm, pathogens can cause chronic infections such as lung infections, urinary stent infections, prosthetic joint infections, chronic wounds, and burn-related infections (Marcinkiewicz *et al.*, 2013).

These chronic infections differ from colonization in that they are characterized by an immune response from the host and persistent pathology (Høiby *et al.*, 2010).

A. baumannii forms biofilms on both biotic and abiotic surfaces which contributes to its remarkable ability to survive in hospital environments. While extrinsic factors such as surface hydrophobicity, temperature, and oxygen concentration are reported to influence *A. baumannii* biofilms, numerous physicochemical and microbial features (e.g., capsular polysaccharides, surface appendages, adhesins, and virulence and resistance determinants) facilitate the formation and maintenance of *A. baumannii* biofilms (Eze *et al.*, 2018).

The ability of *A. baumannii* to form biofilms on abiotic surfaces depends on the production of pili. OmpA also participates in the development of biofilms on plastic surfaces (Poirel and Nordmann, 2006).

Biofilm-associated protein (Bap) is a surface-exposed, highly divergent protein that is required for adherence to bronchial cells and structural integrity and water channel formation within the biofilm, Another recent study found that the variation in the bap coding sequence across *A. baumannii* lineages results in differential functions during biofilm development with some versions displaying better adherence properties and others forming more complex biofilms (Brossard and Campagnari, 2012).

A. baumannii have one quorum sensing (QS) system, which plays an integral role in regulating virulence factors, biofilm formation and surface motility. AbaI is the autoinducer synthase that generates the QS molecule N-(3-hydroxydodecanoyl)-L-HSL ,acyl homoserine lactones (AHL), which at high enough density interacts with the cognate receptor AbaR leading to downstream cellular responses (Subhadra *et al.*, 2018).

A. baumannii frequently causes biofilm-related infections, particularly ventilator-associated pneumonia and catheter-related infection, which can be exceedingly resistant to antibiotic therapy, offering a severe challenge to the clinical management of *A. baumannii*-related biofilm infections. *A. baumannii* biofilms have become one of the most serious

global issues due to the rapid spread of medical device-associated infections and antibiotic resistance. Despite these problems causes, understanding the magnitude of *A. baumannii* biofilm formation; its role in pathogenesis and antimicrobial resistance is important for limiting medical device-associated infections (Gedefie *et al.*, 2021).

1.3.8 The role of Nanoparticles against *A. baumannii*

Nanoscience and nanotechnology have attracted a great interest over the last few years due to its potential impact on many scientific areas such as energy, medicine, pharmaceutical industries, electronics, and space industries. This technology deals with small structures and small-sized materials of dimensions in the range of few nanometers to less than 100 nanometers. Nanoparticles (NPs) show unique and considerably changed chemical, physical, and biological properties compared to bulk of the same chemical composition, due to their high surface-to-volume ratio. NPs exhibit size and shape-dependent properties which are of interest for applications ranging from biosensing and catalysts to optics, antimicrobial activity, computer transistors, electrometers, chemical sensors, and wireless electronic logic and memory schemes. These particles also have many applications in different fields such as medical imaging, nanocomposites, filters, drug delivery, and hyperthermia of tumors (Bayda *et al.*, 2020) .

An important area of research in nanoscience deals with the synthesis of nanometer-size particles of different morphologies, sizes, and monodispersity . In this regard, there is a growing need to develop reliable, nontoxic, clean, ecofriendly, and green experimental protocols for the synthesis of NPs (Iravani *et al.*, 2014).

One of the options to achieve this objective is to use natural processes such as use of enzymes, microbial enzymes, vitamins, polysaccharides, biodegradable polymers, microorganisms, and biological

systems for synthesis of NPs. One approach that shows immense potential is based on the biosynthesis of NPs using bacteria (a kind of bottom-up approach) (Iravani *et al.*, 2014).

The antimicrobial activity of NPs depends on several physicochemical properties, such as their size, shape, solubility, and ability to form free biocidal metal ions (Gold *et al.*, 2018).

Generally, smaller NPs show increased antibacterial activity compared to larger NPs (Abdussalam-Mohammed, 2020). Gram-positive and Gram -negative bacteria differ in terms of cell membrane components and structures and have different adsorption pathways for NPs . The susceptibility of bacteria to NPs depends on their biochemical composition since different NPs target different biomolecules (da Silva *et al.*, 2019).

Both organic and inorganic nanoparticles are reported to have antibacterial and anti-biofilm potencies (Mohid *et al.*, 2019).

These are also used as surface-coating and drug-delivery agents and thus offer a very promising alternative to conventional methods of biofilm control (Fam *et al.*, 2020).

It is important to note that in vivo testing of nanoparticles has only been pursued with planktonic *Acinetobacter* (Vairo *et al.*, 2020). This is dependent on the constituents, type of surface and coating materials. For example, surface modification of titanium implants through doping with silver and/or gallium enhances antibacterial effectiveness against MDR *A. baumannii* (Gulati *et al.*, 2021).

Conventional drugs are losing their functional value due to the rapidly developing drug resistance in microorganisms. This insusceptibility prompted researchers to exploit nanoparticles as an alternative approach to deal with aggressive pathogens like *Acinetobacter*. Multiple mechanisms

have been reported to explain the bactericidal action of nanoparticles, they can penetrate extracellular polymeric substances (EPS) , disrupt cellular morphology, inactivate vital enzymes and proteins, denature proteins, generate Reactive oxygen species (ROS), inhibit DNA replication and prevent ribosome interactions (Godoy-Gallardo *et al.*, 2021).

Such multi-mode bactericidal action of nanoparticles is beneficial since bacteria would have to develop a number of mutations simultaneously to survive (Wang *et al.*, 2021) .

However, this raise concerns on the specificity of nanoparticles to kill a particular pathogen. Unlike traditional antibiotics, inherited resistance towards organic and inorganic nanoparticles has not been observed in bacteria. However, a recent study suggested that bacteria could evolve to acquire resistance through genetic mutations on continuous treatment with AgNPs for 225 generations. Hence, care should be taken to avoid unintentional and unnecessary exposure of microorganisms towards these nanoparticles (Graves Jr *et al.*, 2015).

1.3.8.1The effect of silver-doped titanium dioxide(Ag-TiO_2)Nanoparticles against *A. baumannii*

Matsunaga and co-workers has proved that TiO_2 has antibacterial properties originated from oxidation of bacteria (Mastunaga, 1985). After that, Tio_2 has been used in a wide range of application such as photocatalyst, nanomedicine, textiles, materials devices, water sanitization, control plant disease, wound theraphy, bactericidal application (Díez-Pascual, 2020). Tio_2 photocatalyst is the most frequently used material in transforming toxic organic molecules to H_2O, CO_2.

Silver has been doped in Tio_2 nanosolution because silver in ionic or nanoparticle forms has high antimicrobial activity (Ahmad Barudin *et al.*, 2013).

Ag-Tio_2 nanoparticles destroy microbes and bioaerosols (dust mites, mold spores) by disintegrating their DNA. Silver nanoparticles may directly attach to and penetrate the cell membrane to kill spores, although penetration of silver nanoparticles into microbial cell membranes is not completely understood (Jafari *et al.*, 2018).

The phase structure, crystallite size and crystallinity of Tio_2 play an important role in antibacterial activity. Many studies have confirmed that the anatase phase of titania shows higher antibacterial and antifungal activity than the brookite or rutile phase (Moradpoor *et al.*, 2021).

1.3.8.2The effect of single-wall carbon nanotubes (SWCNTs-OH)Nanoparticles against *A. baumannii*

Carbon nanotubes (CNTs) represent one of the best examples of novel nanostructures , exhibit a range of extraordinary physical properties, such as extremely small size and high aspect ratio (>1000), a rich spectrum of extraordinary electrical, thermal and mechanical properties (Abo-Neima *et al.*, 2020, Alim *et al.*, 2018).

Also, CNTs exhibit a strong antimicrobial activity and can pierce bacterial cell walls (Abo-Neima *et al.*, 2020). These properties allow CNTs to be one of the most prospective materials for use in various fields of science and technologies, such as nanoscale electronic devices , tissue engineering, polymer composites and wastewater treatment filters (Abo-Neima *et al.*, 2020).

CNTs have attracted increasing attention for their potential applications in the biomedical field as diagnostic and therapeutic nano-

tools, so that the effects induced by CNTs, on the biological environment is considered one of the important research fields in the world of biomedical applications of nanoparticles (Abo-Neima *et al.*, 2020).

The antibacterial activity of CNTs influenced by the same factors that usually affect the behaviors of CNTs when in contact with different cell types: their diameter, length, aggregation, concentration, surface functional groups, buffer solution as well as contact time, intensity and the cell type(Yu *et al.*, 2018) (Abo-Neima *et al.*, 2020).

In recent studies, the treatment of *Acinetobacter* infections was possible with several antibiotics like beta-lactams, aminoglycosides, and tetracycline, but now resistance to all known antibiotics is visible in *A. baumannii*. A new therapeutic strategy presented in the world and one of the most important and least costly procedures is the use of nanoscience in the treatment of diseases or the addition of nanomaterials in hospital disinfectants. Nowadays, nanoparticles, especially carbon nanotubes, are used to treat infections as well as cancers (Yazdani *et al.*, 2021).

The main principle of the bactericidal action of CNTs is achieved by combining physical and chemical mechanisms (López de Dicastillo *et al.*, 2018). Physically, CNTs may be the cause of substantial structural damage to the cell wall and membrane of the microorganism. In addition, they have the ability to biologically isolate cell from their microenvironments, leading eventually to the production of toxic substances, such as reactive oxygen species, and placing the cell under oxidative stress leading to the biological death (Abo-Neima *et al.*, 2020).

1.3.9 Genotyping of *A. baumannii*

For bacterial typing to be useful, the development, validation and appropriate application of typing methods must follow many criteria. The ability to quickly and reliably differentiate among related bacterial isolates is essential for epidemiological surveillance systems. There are several typing methods used in laboratories today (Van Belkum *et al.*, 2007).

The methods for bacterial phenotyping have a clear purpose in the confirmation and clarification of local and national healthcare-associated outbreaks due to bacterial strains these range from methods based on simple phenotypic features to DNA sequencing. Previously, the comparison of phenotypic characters, such as colony morphology, color, odor, antibiogram-based typing, biotyping, serotyping and the ability to grow in the presence of specific substances, were used for differentiation. Today these methods are becoming old because they require strict standardization of experimental conditions since phenotypes are quite susceptible to the environmental conditions (Monem *et al.*, 2020).

Microbial genotyping technologies are a significant tool for molecular epidemiology researches, especially in understanding pathogen transmission and population dynamics. Despite the prevalence of whole-genome data, multilocus sequence typing (MLST) remains the “gold standard” for molecular typing of bacteria (Cinar *et al.*, 2020).

Molecular typing is becoming a paradigm for understanding the fundamental mechanisms of *Acinetobacter* infections in hospital settings to investigate the spreading, the clonality relationship among bacterial strains and its geographical spread. In infection control, molecular typing methods are currently an important tool to measure and identify the source of the original infection in hospitals outbreaks. Molecular typing methods have been used to investigate the nosocomial spread of *A. baumannii* outbreaks

in hospital settings worldwide. Some examples of these techniques are plasmid typing, ribotyping, pulsed field gel electrophoresis (PFGE) and polymerase chain reaction (PCR) based fingerprinting. As compared with other methods, PCR based fingerprinting methods are easier to perform and cost effective (Aljindan *et al.*, 2018).

The presence of tandem DNA repeats in genomes of *A. baumannii* was confirmed by several groups(Villalón *et al.*, 2015) Based on these sequences, different methods of differentiation of *A. baumannii* strains have been developed; however, they take into account only their diversifying power of evolutionary changes of the *Acinetobacter* genus. Thus, their features responsible for drug resistance or pseudo-immunological bacterial responses, encoded in the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system, which evolved to protect the cells from exogenous phage and plasmid DNA invasion, are ignored in such analyses. On the other hand, as suggested by (Abby *et al.*, 2014), the next step in the process of strains' classification should be focused on confrontation of the genetic and phenotypic features related to pathogenicity of bacterial species, It is based on combination of the previously described method based on analysis of repeated sequences and whole genome alignment (Kotłowski *et al.*, 2021).

The bacterial genome of *A. baumannii* contains recurring sequences similar to the enterobacterial repetitive intergenic consensus (ERIC) initiator sequence. This has enabled it to be used as a molecular biological tool for determining the genetic variation coding on DNA of *A. baumannii* isolates (Mohammed and Ahmad, 2021).

Rapid amplification of polymorphic DNA (RAPD) This is one of the easiest genotyping methods to evaluate strain-level fingerprinting, using PCR and consequently performing gel electrophoresis. No

knowledge of the DNA target sequence is required, as a primer anchors to a non-specific site in the DNA sequence. The primer target sites may vary in number and location over the genome; accordingly, the size and number of the amplified fragments differ. Usually, decamer (10 nucleotides) lengths of non-specific short sequence primers are used under low annealing temperature, and electrophoresis RAPD profiles can be used for strain genotyping. Various primers and protocols have been used to type the *Acinetobacter* species (Ahmed and Alp, 2015).

A melting curve analysis was performed for the amplified DNA fragments that were generated during RAPD. This approach does not require electrophoresis and ethidium bromide by real-time PCR, thus greatly reducing workload and overall time (Deschaght *et al.*, 2011).

RAPD has many advantages: it requires low-cost primers, only a small quantity of DNA for analysis, and it requires no blotting or hybridization. However, the introduction of commercial and standardized reagents made RAPD fingerprinting more time consuming to standardize. These patterns are strain specific; RAPD could therefore be a useful approach for epidemiological genotyping (Ahmed, 2017).

Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) is one of the best typing methods of DNA fingerprints of bacterial strains. This method has sporadically been used to characterize *A. baumannii* strains isolated from clinical samples (Nemec *et al.*, 2004).

In *A. baumanni* , repetitive sequences of the gene which are called symmetric elements are often seen in the non-coding fragment of the DNA. Regarding the variable number and length of these repeat sequences, some primers have been designed. The length and number of bands obtained for

each isolate are variable and the strains could be grouped according to the diversity of the bands (Maleki *et al.*, 2018).

Bacterial genomes containing Repeat sequences such as the ERIC sequence can be used as Molecular biological tools to assess the clonal variability of Many bacterial isolates. ERIC-PCR fingerprinting is one of the fastest molecular typing techniques to differentiate Between *A. baumannii* and other strains of Gram-negative Bacteria responsible for hospital-acquired infections (Aljindan *et al.*, 2018).

BOX elements are mosaic repetitive elements comprised of different combinations of three subunit sequences. These three subunits sequences are boxA, boxB, and boxC which are 59, 45, and 50 nucleotides long, respectively. Meanwhile, ERIC sequences are 126 bp long with a highly conserved central inverted repeat. It is situated in noncoding transcribed regions of the chromosome. Briefly, the BOX primer anneals on the boxA subunit of BOX elements whereas the ERIC primer synthesizes DNA sequences outward from inverted repeats (Bilung *et al.*, 2018).

1.3.9.1 Clustered regularly interspaced short palindromic repeat (CRISPR)

Clustered regularly interspaced short palindromic repeat (CRISPR) arrays and CRISPR-associated genes (*cas*) constitute bacterial adaptive immune systems and function as a variable genetic element. CRISPRs are bacterial loci whose dynamic nature has allowed them to become ideal targets for molecular subtyping. Each CRISPR/*Cas* locus includes a strain-specific array of spacers that has expanded and diversified over time. Due to their dynamic nature, comparative analysis of the spacer arrays has successfully been used for subtyping isolates from several Gram-positive and Gram-negative bacteria (van Belkum *et al.*, 2015).

Two CRISPR/Cas systems have been found in the genomes of several *A. baumannii* strains (Karah *et al.*, 2015). Studies have reported that trailer end spacers of CRISPR are generally conserved among different isolates and can be used to anchor clusters and detect common ancestors of the arrays and, probably, of the isolates themselves (Horvath *et al.*, 2008). More and more data are accumulating indicating that the role of CRISPR/Cas is not limited to adaptive immunity. It has been shown that these systems regulate the expression of many bacterial genes affecting the virulence of pathogenic bacteria and group behavior, and also participate in DNA repair and accelerate the evolution of genomes(Tyumentseva *et al.*, 2021)

Complete CRISPR-cas loci consist of a CRISPR array, that is, two to several hundred direct, often partially palindromic, normally exact repeats (25–35 bp each), separated by unique spacers (typically 30–40 bp each), and the adjacent cluster of multiple *cas* genes which are organized in one or more operons encoding both the adaptation and the effector modules, often along with accessory genes (Makarova *et al.*, 2013) . The CRISPR-*Cas* immune response includes three distinct but often intertwined stages: (i) adaptation, (ii) pre-crRNA (pre-CRISPR RNA) expression and processing, and (iii) interference (Koonin and Makarova, 2019).

the CRISPR-*Cas* system protects against mobile genetic elements (MGEs), thereby defending against viral predation, The complexity of the CRISPR-*Cas* system warranted a comprehensive classification of the system. This was achieved by basing the classification on phylogenetic analysis of conserved cas proteins, type and number of *cas* genes and gene arrangements (Makarova and Koonin, 2015). To date, four types and 12 subtypes (excludes six variant subtypes) of CRISPR-Cas system have been reported (Sykes *et al.*, 2020).

The target recognition by a CRISPR-Cas system depend on the Cas protein binding Protospacer Adjacent Motif (PAM), leading to unwinding of the adjacent dsDNA helix (Jones *et al.*, 2017). The RNA-directed aspect of the CRISPR-Cas is dependent on two RNAs, the CRISPR RNA (crRNA) and a *trans*-acting RNA (tracrRNA). The tracrRNA, which imparts partial complementarity, and the crRNA, can be combined to form a single guide RNA (sgRNA), that performs DNA target recognition and recruits *Cas* nuclease needed to perform genome editing. The substrate specificity and the simplicity of the system that includes designing sgRNA makes CRSIPR an obvious choice for genetic manipulation. Following unwinding of the dsDNA, the single guide RNA (sgRNA) hybridizes to form a R-loop structure. This allows recognition of seed sequence near PAM elements enabling complementarity with the crRNA spacer and the CRISPR-Cas system to execute its effect (Jones *et al.*, 2017).

The spacer organization in CRISPR-*cas* have been used for comparison between strains. A pangenome study encompassing 2,500 genomes of *A. baumannii*, agreed to the presence of two CRISPR-Cas systems (Mangas *et al.*, 2019). By constructing a new phylogeny of *Acinetobacter* based on presence or absence of 14 common genes, they divided the genomes in to two groups. The first group, sharing fewer common genes, showed gene enrichment for maintaining CRISPR elements and rarity in the presence of plasmids. The relationship between CRISPR-*cas* and virulence genes have been discussed in a study (Louwen *et al.*, 2014). With the success of endogenous CRISPR-Cas system including type I in genome editing in multiple bacteria strains, the same may be explored in *A. baumannii*. In addition, useful information would be obtained by better understanding of the relationship of CRISPR-Cas systems in *A. baumannii* with the virulence and pathogenesis (Sykes *et al.*, 2020).

Chapter Two

Materials and Methods

2.1 Materials

2.1.1 Equipment and Instruments

Equipment and instruments used in the study were listed in table 2-1.

Table 2-1: Equipment and Instruments Used in the study.

Equipment	Manufacturing Company/ Origin
Autoclave	Jeiotech/ South Korea
Centrifuge	Hettich / Germany
Cooling Centrifuge	Hettich /Germany
Deep-freezer	Hicool/ Denmark
Digital camera	Canon/ Japan
Electrical balance	KERN/ Germany
Eppendorf tubes	Sterellin Ltd /UK
Hot plate with magnetic stirrer	IKA/ Germany
Incubator	Memmert/ Germany
Light microscope	Olympus /Japan
Microcentrifuge	Hettich/ Germany
Microtiter plate (96) flat- shape	Sterilin Ltd/ UK
Millipore filter unit (0.22 µm)	Schleicher and Schuel / USA
Nanodroop	Avans/UK
PCR Thermocycler	Clever/ England
Refrigerator	Crafft/ Saudiaarabia
Safety cabinet (HOOD)	Gallenkamp/ USA
Spectrophotometer	Shimadzu/ Japan
Vitek 2 compact	Biomerieux/ France
Vortex	Fanem/ Brazil
Water bath	Memmert/ Germany
Water distiller	Paytekht Co.Ltd/ Iraq

2.1.2 Chemical and Biological Materials

Chemical and biological materials used in this study were summarized in the table 2-2:

Table 2-2: Chemical and Biological Materials Used in this Study.

Chemical and biological material	Manufacturing Company/ Origin
Agarose	Promega/USA
$AgTio_2$ nanoparticales	Hongwu International Group Ltd/ china
Ethanol 70%,obsolute	BDH / UK
Ethidium bromide	Promega/USA
Glucose	Oxoid / UK
Glycerol	BDH / UK
Gram Stain kit	HiMedia/ india
Hydrogen peroxide (H2O2)	BDH / UK
N-N-N-Ntetramethyl-P-phenylene diamine dihydrochloride	Hi-Media/ India
Normal Saline	PSI/ Saudi Arabia
Nuclease Free Water	Promega/USA
Phosphate buffer saline (PBS-pH=7. 2)	BDH/ England
Selective supplement (FD271)	HiMedia/ India
SWCNT-OH nanoparticales	Hongwu International Group Ltd/ china
Tris-Borate-EDTA (TBE) buffer	Promega/USA
Tris-EDTA buffer (TE)	Promega/USA

2.1.3 Culture Media

All the culture media were used in this study are listed in table 2-3.

Table 2-3: Culture Media Used in this Study

Media	Manufacturing Company/ Origin
Blood agar base	Himedia /India
Brain heart infusion broth & agar	Himedia /India
CHROMagar™ *Acinetobacter*	Himedia /India
MacConkey agar	Oxoid / England
Mueller Hinton agar	Oxoid / England
Nutrient broth & agar	Oxoid / England

2.1.4 Kits

The diagnostic kits used in the current study were presented in Table 2-4.

Table 2-4: Diagnostic Kit Used in this Study.

Kit	Manufacturing Company / Origin
DNA extraction kit	Geneaid/ USA
DNA ladder 100bp	Bioneer/ Korea
Green master mix 2X Kit	Promega/USA
Vitek 2 System	Biomerieux/ France

2.1.5 Antibiotic Disks

The antibiotic used in the current study for each isolate was determined and recommended by CLSI, were presented in Table 2-5.

Table 2-5: Antibiotic Discs Used in this Study

Antibiotic	Code	Disk potency (µg/disc)	Manufacturing Company /Origin
Amoxicillin- clavulanic acid	AMC	20/10	Mastdiscs / UK
Ceftazidime	CAZ	30	
Cefepime	FEP	30	
Meropenem	MRP	10	
Gentamicin	GEN	10	
Amikacin	AK	30	
Ciprofloxacin	CIP	5	
Levofloxacin	LEV	5	
Trimethoprim-sulfamethoxazole	SXT	25 1.25/23.75	
Tetracycline	TE	10	

2.2 Methods

2.2.1 Reagents and Solutions

2.2.1.1 Reagents

A- Catalase Reagent

It was prepared by adding 1ml of 30% Hydrogen peroxide to 9 ml of D.W. thefinal concentration of the solution was 3% and then it was kept in a dim bottle (Procop *et al.*, 2017). This reagent was used for detecting capability of bacteria isolates to produce catalase.

B- Oxidase Reagent

It was prepared by dissolving 1gm of N-N-N-N tetramethyl-P-phenylene diamine dihydrochloride in 100 ml of distilled water and kept in

a dark bottle in refrigerator. This solution was used to detect the capability of bacterial isolates to produce oxidase (Shields and Cathcart, 2010)

2.2.1.2 Solutions Buffer

It has been prepared by dissolving one Buffer tablet in 100 ml D.W.and sterilized by autoclaved according to the instruction of manufacturer,after that was kept at 4ºC until used.

2.2.2 Preparation of Culture Media

2.2.2.1 Ready-Culture Media

Ready-made culture media were prepared according to the manufacturing company instructions, while the other culture media were prepared in the laboratory according to the scientific references. All these media (with some exceptions) wereautoclaved at 121°C for 15 minutes at 15 pound per square inch (Brown and Smith, 2014).

2.2.2.2 Laboratory Prepared Culture Media

A- Blood Agar

According to the manufacturer company, Blood agar base was prepared. Thenwas autoclaved and cooled to (45-50)ºC, aseptically adding 5 ml of sterile human blood for each 95 ml of the medium mixing well to homogeneity, distributed into sterile Petri dishes. Finally, the prepared medium was cooled to 37ºC and was left to solidify at room temperature (25ºC). (Harley and Prescott, 1996).

B- MacConkey Agar (PH: 7.3)

This media was prepared according to the instruction of the industrialized company. McConkey agar is used to isolate most Gram - negative bacteria and used to differentiate between lactose fermenter and non-fermenter bacteria.

C-Nutrient Agar Medium

This nutrient agar was prepared according to the instruction of supplier company by dissolving 28gm of nutrient agar in 1L of distilled water then sterilized in the autoclave at 121 Cº for 15 min. , this medium was used in general experiment such as cultivation and activation of bacterial isolates when it necessary.

D- Müller-Hinton Agar

This medium was prepared according to the instruction of supplier company by dissolving 38gm from the medium in 1L of distilled water and sterilization by autoclave at 121 Cº for 15 min., this medium used for an antibiotic sensitivity test.

E- Brain Heart Infusion (BHI) Broth -Glycerol Medium(Maintenance Medium)

This medium used to preserve the bacterial isolated as the standard for a long time. This medium was prepared by adding 5ml of glycerol with 95 ml of BHI broth and sterilization by autoclave at 121 Cº for 15min. (Forbes *et al.*, 2007).

F- CHROMagar™ *Acinetobacter*

CHROMagar (HiCrome TM) Acinetobacter was prepared according to the manufacture company instructions suspend 30.85gram in 1000ml D.W heated to boiling while swirling until completefusion of the agar then cooled to 45-50°C with adding Multidrug resistance selective supplement (FD271) and poured into sterile Petri dishes. Finally,it was cooled to 37ºC and left to solidify at room temperature then used for specificscreen and identification of *A. baumannii* to form light purple color with halos rounded the colonies on it.

G- Motility Semi-Solid

This medium was prepared according to Hargrave, (2000) by mixing: peptone water (10g), sodium chloride (5g), agar (3.5g) and D.W. (1000 ml). The prepared medium was first dispensed in 900 ml distilled water then pH was adjusted to 7-7.4and the volume was completed to one liter, then 10 ml from this medium was dispensed in each sterile vial then autoclaved at 121ºC and 15 pound/inch2 for 15
minutes. Finally cooled to 37ºC and left to solidify at room temperature (25ºC) thenused for identifying motile and non-motile bacteria.

2.2.3 Ethical approval

The necessary ethical approval was taken from the ethical committee in Al-Imam Al-Sadiq Hospital and Babil Teaching Hospital, Moreover, agreement from the family and patients for sampling and carrying out this work was obtained.

The College of Medicine Ethics committee approves the research proposal to be conducted in the presented form. None of the Investigator and co-investigator participating in this study took part in the decision making and voting procedure for this study.

The College of Medicine Ethics committee expects to be informed about the progress of the study, any serious adverse events occurring in the course of the study, any revision in the protocol.

2.2.4 Study design

Cross-sectional study.

The design of study was illustrated in figure 2-1.

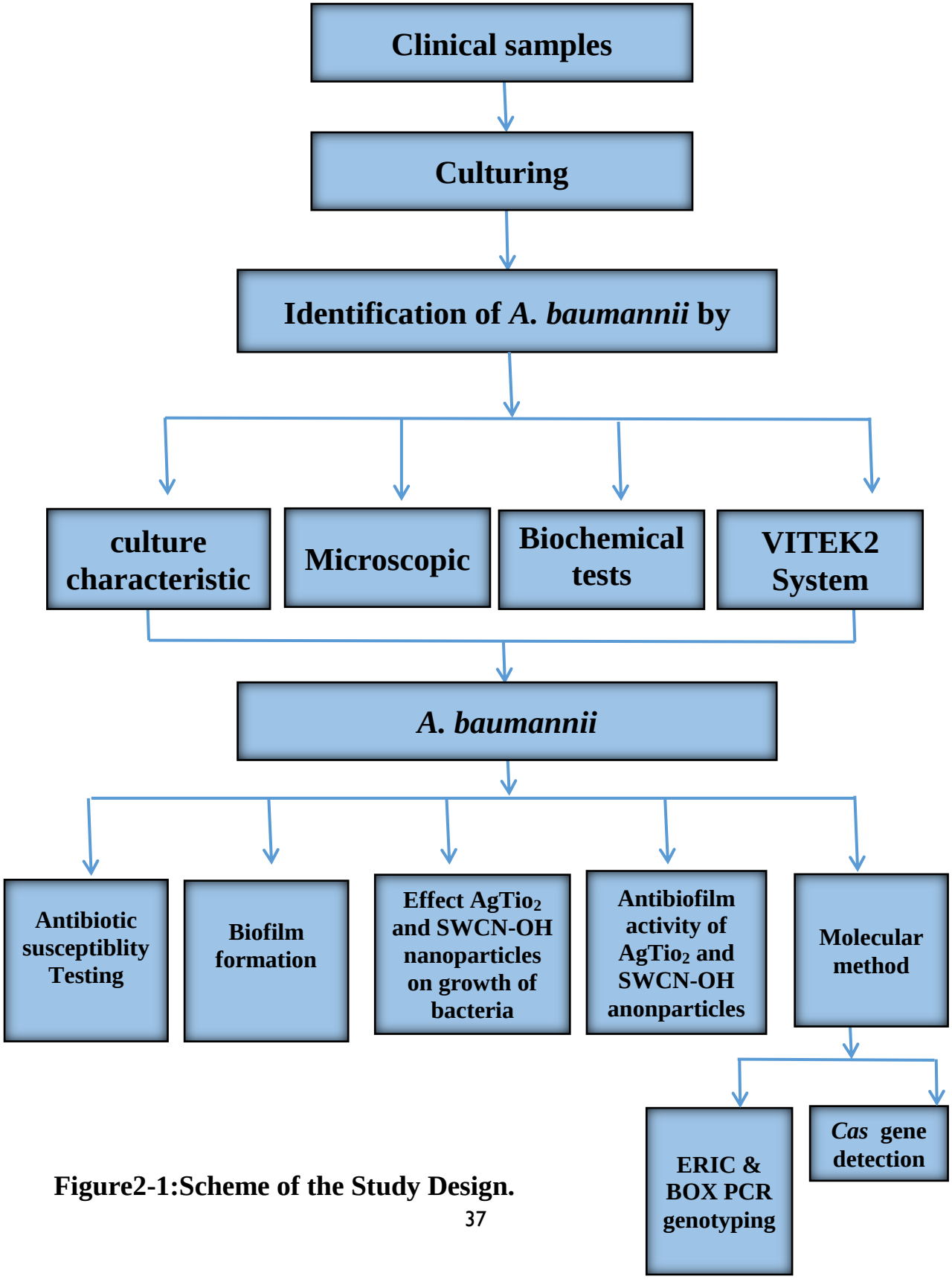

Figure2-1:Scheme of the Study Design.

2.2.5 Clinical specimens' collection

One hundred and fifty clinical specimens were collected during the present study period extended from October 2021 to January 2022 from patients who were attended to two main hospitals in Hilla city: Al-Imam Al-Sadiq Hospital and Babil Teaching Hospital suffering from different infections as following; burns (35 sample) , wounds (40 sample), respiratory infections (45 sample), and urinary infections (30 sample).

Urine (mid-stream urine) was collected from patients suffering from urinary tract infection (UTIs) in a sterile screw-cap container. Swabs from the burn wound. Sputum. samples were collected from patients before they take any antibiotics or cleaning. The samples were transferred immediately to the laboratory. Each sample was cultured on different media including MacConkey and nutrient agar then incubated aerobically at 37 Cº for 24 hours.

2.2.6 Isolation of Bacteria

In the laboratory and under aseptic conditions, the collected sample were re- identified by culturing directly on blood agar and MacConkey agar, and all the isolates were incubated for 24 hours at 37ºC. The non-hemolytic opaque creamy colonies on blood agar and non- lactose fermenting on MacConkey agar, sub- cultured on another MacConkey agar plate and were incubated for another 24 hours at 37ºC to obtain pure well isolated colonies (Bailey and Scott, 1962) (Forbes *et al.*, 2007). Also, a specific and rapid isolation of *A. baumannii* was conducted based on CHROMagar (HiCrome TM) with adding Multidrug resistance selective supplement (FD271). All isolates of growth including (MDR) appeared within (24 to 48 h) at 37°C, as light purple color with halos rounded the colonies. The positive control of *A. baumannii* (ATCC, 19606) was

cultured and the results compared with all isolates to give 100% consistency.

2.2.7 Bacterial Identification

2.2.7.1 Microscopic Examination (Gram Stain)

One isolated colony was transferred to a microscopic slide, fixed then stained with Gram stain, cell shape and arrangement were recorded. The results were compared with (Brooks *et al.*, 2013).

2.2.7.2 Biochemical Tests

All the biochemical tests were carried out according to (Brown and Smith, 2014)

A- Catalase Test

On a clean microscope slide this test was performed by using the end of a wooden swab, some cells from the bacterial culture were transferred to the surface of a clean microscope slide, two to three drops of 3% hydrogen peroxide (2.2.1.1.A)were added to the cells, then were mix with the wooden stick, and vigorous bubbleswere observed.

B- Oxidase Test

A filter paper was saturated with little drops of 1%N-N-N-N-tetramethylpara- phenylenediamine dihydrochloride (2.2.1.1.B). With a woody applicator growth from an agar medium was smeared on the paper. A positive check was formed a purple color within 10 seconds.

C- Motility Test

Motility of bacterial isolates was checked in semi solid motility medium (2.2.2.2.G). Cultures were inoculated with a loop needle into the center of the tube and incubated at 37°C for 24 hr. movement away from the stab line or a cloudy appearance around the stabbing area indicated a motile organism.

D- Hemolysin Production

This test was used to detect hemolysin production on blood agar previously prepared as mentioned in section 2.2.2.2.A. The plates were cultured with the tested bacteria by streaking method and incubated at 37˚C for 24 hours. A hemolysis around the colony should be observed for the positive result.

2.2.7.3 Vitek 2 system diagnosis

The Vitek 2 System was used to confirm the result of the manual biochemical test, this system used to identify microorganisms . This system was performed according to the manufacturer's instructions (Biomerieux-France).

This system consists of :

1- A personal computer.

2- Reader/incubator that consisting of multiple internal components including: card cassette, card filler mechanism, cassette loading processing mechanism, card sealer , bar code reader, cassette carousel and incubator.

3- The system also contains: transmittance optics , waste processing, instruments control electronics and firm ware. This system was performed according to the manufacturer's Instructions (Biomerieux-France):

1- Three ml of normal saline were placed in plane test tube and inoculated with a loopfull of single colony of overnight culture.

2- The test tube was inserted into a dens check machine for standardization of colony to McFarland's standard solution (1.5×10^8 CFU/ml).

3- The standardized inoculums were placed into the cassette.

4- Then a sample identification number was entered into the computer software via barcode . Thus the vitek 2 card was connected to the sample ID number. The cassette was placed in the filler module, when the cards were filled, transferred the cassette to the reader/incubator module.

2.2.8 Preservation of Isolates

2.2.8.1 Short Term Preservation

Bacterial isolates were maintained for few weeks on nutrient agar plates which were wrapped tightly with parafilm and then stored at 4ºC(Harley and Prescott, 1996) .

2.2.8.2 Long Term Preservation

Brain heart infusion broth addition to 5% glycerol was distributed into plain tube, autoclave and inoculated with 24 hr. ancient culture of bacteria isolates as wellas incubated at 37°C for 24 hr. then stored at -20°C (Vandepitte *et al.,* 2003).

2.2.9 Antibiotic Susceptibility Test

Kirby -Bauer method used to perform carry out the antibiotic susceptibility test for 10 different antimicrobial agent Bacterial suspension was prepared by picked 4-5 colonies of each bacterial isolate from original culture and was suspended into a test tube containing 5 ml of normal saline, then turbidity was adjusted to obtain approximately 1.5 $x10^8$ CFU/ml (MacFarland tube). By a sterile cotton swab a portion of bacterial suspension was transferred carefully and evenly spread on Mueller - Hinton agar medium, and then it was left for 10 min. Then after the antimicrobial discs were placed on the

agar with sterile forceps pressed firmly to ensure contact with the agar. Later the plates were inverted and incubated at 37 C° for 24 hr. Inhibition zones that developed around the discs were measured by millimeter (mm) unit by using a metric ruler. The isolate was interpreted as susceptible, intermediate, or resistant to particular antibiotic according to CLSI 2020(Clinical and M100, 2020).

2.2.10 Biofilm formation

2.2.10.1 Tissue culture plate method (TCP)

Organisms isolated from fresh agar plates were inoculated in 10 mL of BHI broth with 1% glucose. Broths were incubated at $37C^0$ for 24 hrs. The cultures were then diluted 1:100 with fresh medium. Individual wells of sterile 96 well- flat bottom polystyrene tissue culture-treated plates were filled with 200µL of the diluted cultures. The control organisms were also incubated, diluted and added to the tissue culture plate. Negative control wells contained inoculated sterile broth. The plates were incubated at 37^oC for 24 h. After incubation, contents of each well were removed by gentle tapping. The wells were washed with 0.2 mL of phosphate buffer saline (pH 7.2) four times. remove free-floating bacteria. Biofilm formed by bacteria adherent to the wells were fixed by 2% sodium acetate and stained by crystal violet (0.1%). Excess stain was removed by using deionized water and plates were kept for drying. Optical density (OD) at 570nm of stained adherent biofilm was obtained by using Spectrophotometer. The experiment was performed in triplicate and repeated three times, the data were then averaged, and the results were interpreted according to the table (2-9) (Oliveira and Maria de Lourdes, 2010).

Table (2-6)Tissue Culture Plate Method (TCP)

Mean OD values	Biofilm formation
<0.120	Non
0.120-0.240	Moderate
>0.240	High

2.2.11 Preparation of nanoparticles suspension

2.2.11.1- Preparation of SWCNT-OH nanoparticles suspension:

Ten mg of SWCNT-OH nanoparticles ,the size of SWCNT is 50 nm, dissolves in 10 ml Polyethylene glycol (PEG)-400 solution (as a surfactant) to prepare a suspension of 1000µg/ml , continuous ultra-sonication (over-night) for the suspension was achieved at the time of preparation and each time prior to use so as to re-disperse the particles in the solution (Kharisov *et al.*, 2013).

2.2.11. 2- Preparation of $AgTio_2$ nanoparticles suspension:

Ten mg of $AgTio_2$ nanoparticles ,the size of $AgTio_2$ is 20nm, dissolves in 10 ml D. W to prepare a suspension of 1000µg/ml , continuous ultra- sonication(over-night) for the suspension was achieved at the time of preparation and each time prior to use so as to re-disperse the particles in the solution (Kharisov *et al.*, 2013).

2.2.12 The effect of $AgTio_2$NPs and SWCNT-OH on Bacterial Growth at 24 and 48-hours Incubation time

In brief, first well contain only 300 µl of BHI broth to adopt it as negative control while the remaining wells were filled with 150 µl of bacterial suspension except positive controls well which is filled with double volume. Subsequently, the $AgTio_2$ (150 µl) was added to the third wells. Then, double fold serial dilution was carried out across the plate beginning with 1/2,1/4,1/8,1/16 and finally 1/32 dilution all dilution wells with duplicated ,then the plates were incubated for 24 hours and 48 hours at 37°C.

The same steps mentioned above were repeated, but by adopting SWCN- OH as an anti-growth instead of $AgTio_2$.

the effect of Ag Tio2 and SWCN- OH Nanoparticles was read by ELISA reader at 405 nm. For 48 h the experiment was applied with the same conditions used in 24h experiment but the incubation period was 48 h (Mubdir *et al.*, 2021).

2.2.13 The effect of $AgTio_2$NPs and SWCN- OH on Bacterial Biofilm production:

The antiadhesion properties of $AgTio_2$ against *A. baumannii* strains were tested following a microplate biofilm assay.

Organisms isolated from fresh agar plates were inoculated in 10 mL of BHI with 1% glucose. Broths were incubated at 37oC for 24 h. The cultures were then diluted 1:100 with fresh medium. First wells of sterile 96 wells flat bottom polystyrene tissue culture treated plates were filled with 300 µL of the diluted cultures. The other wells were filled with 150 µl of bacterial suspension, followed by the addition of 150 µl of $AgTIO_2$ stock solution to the first well followed by transferring by double serial dilution. Negative control wells contained clear broth. The plates were incubated at 37^{oC} for 24 h. After incubation, contents of each well were removed by gentle tapping. The wells were washed with 0.2 mL of phosphate buffer saline (pH 7.2) four times. This removed free floating bacteria. Biofilm formed by bacteria adherent to the wells were fixed by 2% sodium acetate and stained by crystal violet (0.1%). Excess stain was removed by using deionized water and plates were kept for drying.

The same steps mentioned above were repeated, but by adopting SWCN- OH as an anti-biofilm instead of $AgTIO_2$.

Optical density (OD) of stained adherent biofilm was obtained by using micro ELISA reader at wavelength 570 nm The experiment was performed in triplicate and repeated three times(Chlumsky *et al.*, 2020).

2.2.14 Genotyping assays of *A. baumanii*

2.2.14.1- DNA Extraction

This method was made according to the genomic DNA purification Kit supplemented by the manufacturing company Geneaid (UK). Chromosomal DNAs obtained were used as templates for all PCR experiments,and according to the following steps:

Step1: Cell Harvesting/pre-lysis

- One ml of bacterial suspension containing approximately up to1.2×10^{9} cell/ml (equal to 4.0 McFarland standard) was transferred to a 1.5ml microcentrifuge tube, centrifugation for 1 minute at 14-16,000×g and discard the supernatant.
- A volume of 200 µl of GT buffer was added to the tube and resuspend the cell pellet by shaking vigorously or pipetting, then left at room temperature for 5 minutes.

Step 2: Lysis

- A volume of 200 µl of GB buffer was added to the sample and mix by shaking vigorously for 5 seconds, then incubated at 70ºC for 10minute
- or until the sample lysate is clear. During incubation, the tube was inverted every 3 minutes. At this time, the required Elution Buffer (200µl per sample) incubated at 70ºC (for step 5 DNA elution).

Step 3: DNA Binding

- A volume of 200 µl of absolute ethanol was added to the sample lysate and immediately mixed by shaking vigorously. If precipitate appears, broke it up by pipetting.
- GD column was placed in a 2ml Collection Tube.
- All of the mixture (including any precipitate) was transferred to the GDColumn and centrifuged at 14-16,000×g for 2 minutes.

- The 2 ml Collection Tube flow-through was discarded and placed theGD Column in s new 2 ml Collection Tube.

Step 4: Washing

- A volume of 400 µl of W1 Buffer was added to the GD Column.
- Then, centrifugation at 14-16000×g for 30 second
- The flow-through was discarded and placed the GD Column back inthe 2 ml Collection Tube.
- A volume of 600 µl of Wash Buffer (ethanol added) was added to theGD Column.
- Centrifugation at 14-16,000×g for 30 seconds.
- The flow-through was discarded and placed the GD Column back inthe 2 ml Collection Tube.

Step 5: DNA Elution

- The dried GD Column was transferred to a clean 1.5 micro centrifuge tube and 100 µl of preheated Elution Buffer was added to the center of the column matrix and let stand for 3-5 minutes or until the Elution Buffer is absorbed by the matrix.
- Centrifugation at14-16,000×g for 30 seconds to elute the purified DNA.

2.2.14.2- Detection of DNA concentration and purity by Nanodroop

The extracted DNA was checked by using nanodrop spectrophotometer, which measured DNA concentration (ng/µL) and check the DNA purity by reading the absorbance at (260/280nm) as following steps:

1. After opening up Nanodrop software, chosen the appropriate application (Nucleic Acid, DNA).

2. A dry wipe was taken to clean instrument pedestals several times. then carefully pipette 2 µl of ddH2O on to the surface of the lower measurement

pedestals for blank system.

3. The sampling arm was lowered and clicked ok to initialized the nanodrop, then cleaning off the pedestals and 1 µl of extracted DNA carefully pipette onto the surface of the lowered measurement pedestals, then concentration and purity of extracted DNA was checked (Wilfinger *et al.*, 1997).

2. 2.14.3 PCR amplification

2.2.14.3.1 Oligonucleotides primer pairs

All primer pairs used in this study were purchased from macrogen (Korea) in lyophilized form. Primarily, the stock solution was prepared by dissolving the lyophilized powder in nuclease free water buffer according to manufacturer's protocol.

2.2.14.3.2 PCR Master Mix

A. Master Mix kit:

GoTaq Green Master Mix kit is a premixed ready-to-use solution for amplification of DNA templates by conventional PCR. This kit was purchased from Promega/USA. This master mix contains the following:

- *Taq* DNA polymerase: This enzyme responsible for the synthesis of new DNA strand during PCR reaction.
- Reaction buffer (2X) important for maintenance, pH about 8.5 also contains a compound that increases sample density.
- Deoxyribotriphosphates (dNTPs) 400 µM of the following: dATP, dGTP, dCTP and dTTP.
- Magnesium ions $MgCl_2$ (3mM), which is a co-factor for enzyme action.
- Loading dye: containing a mixture of yellow dye (xylene cyanol) and blue dye (bromophenol blue), important for direct PCR product loading for analyzed by agarose gel electrophoresis.

B. GoTaq Green Master Mix kit protocol:

The multiplex and uniplex PCR amplification mixture which used for detection of *cas* gene and ERIC/BOX respectively were listed in tables below, table (2-7).

Table (2-7a): Contents of the Reaction Mixture multiplex of PCR

Contents of the reaction mixture multiplex	Volume
Green master mix	12.5µl
Upstream primer	3µl(1 µl for each primer)
Downstream primer	3µl
Nuclease free water	3.5µl
DNA template	3µl
Total volume	25 µl

Table (2-7b): Contents of the Reaction Mixture uniplex of PCR

Contents of the reaction mixture uniplex	Volume
Green master mix	12.5µl
Upstream primer	2.5µl
Downstream primer	2.5µl
Nuclease free water	5µl
DNA template	2.5µl
Total volume	25µl

2.2.14.3.3 Primer Sequences and PCR conditions

The primer sequences and PCR conditions thatused are listed in tables2-8.

Table 2-8: The primer sequences and PCR conditions

Genes Name	Primer sequence (5´- 3´)	PCR condition	Ref.
Cas1	F GCTGCGATGCGAATGTTATGT R AGTACCCAAAGTGTCGTCGC	94°C for 5 min 94°C for 1min 55°C for1min 72°C for1min ***35 cycles** 72°C for10 min	**(Leungtongkam *et al.*, 2020)**
Cas2	F CGCTATGGTGTTTAAGTTACGA R GAAAAACCGCAAGACGGTCA		
Cas3	F ACTTGATTATGATGCTATGCCCA R TCGCTCTTGTTCACTGCGTA		
Cas5	F TGCTTGTGCTTTAGGTGAGCA R CACCGTATGGCTCAATCGCA		
Cas6	F GGAAACTGCCTGCGCATTA R ATCCCGCGTTTTCACTCTCC		
Cas9	F ACTCTCGAAGACAAAGCGCA R TGGTTGCGACCACACAGTTT		
I-F-CRISPR	F GCCTCGAGCCAAGATTACAG R. CCTCACGTTTTGCACTGAGA	94°C for 5 min 94°C for 1min 52°C for1min 72°C for1min ***35 cycles** 72°C for10 min	**design in this Study**
ERIC-1	TGTAAGCTCCTGGGATTCAC	94°C for 5 min 94°C for 1min	**(Aljindan *et al.*, 2018)**

ERIC-2	AAGTAAGTGACTGGGGTGAGCG	52°C for1min 72°C for1min ***35 cycles** 72°C for10 min	
BOX	CTACGGCAAGGCGACGCTGACG	94°C for 5 min 94°C for 1min 40°C for 2min 72°C for 2min ***35 cycles** 72°C for10 min	**(Bilung *et al.*, 2018)**

2.2.14.4 Detection of Amplified Products by Agarose Gel Electrophoresis

PCR products were visualized on 1.5%. Agarose gel electrophoresis stained with Ethidium bromide according to(Lee *et al.*, 2012).

A- Preparation of gel:

- The gel was prepared at a concentration of 1.5% by dissolving 1.5gram of agarose in 100ml of TBE working buffer (1X).
- Heating mixture until agarose was completely dissolved.
- The mixture was left to cool to about 50-60°C then 1 µl of ethidium bromide was added to the gel.

B-preparation of casting horizontal agarose gel:

- The casting platform was placed with well former sideways in gel stand where the gel was poured.
- The gel was poured on an electrophoresis plate fixed on an even surface.
- The comb was placed and the gel was left to cool and solidify for 30 min at room temperature.
- After the gel was set, the combs were removed carefully and the tank was placed into electrophoresis system. Then added 1X TBE buffer until covered gel (approximately 1 - 2 mm above the gel surface).

C-Running of products

- When the thermocycling was finished, the PCR tube was handled outside the thermocycler. 5 µl of each PCR product along with 100bp DNA ladder was loaded into the gel wells.
- The system cover put into place and then turned on. The gel was run for 1hr at 70 volts.

2.2.14.5 Visualization

Following electrophoresis, visualization was conducted with a UV transilluminator and image was captured by the digital camera (Canon, USA).

2.2.14.6 Cluster Analysis

The banding patterns generated by BOX-PCR and ERIC-PCR were analyzed using PyElph version 1.4. The dendrograms were constructed using an unweighted pair group method with arithmetic mean (UPGMA), according to published guidelines by Pavel and Vasile (Pavel and Vasile, 2012). This method was used because UPGMA is the simplest distance-matrix method in constructing a phylogenetic tree using uncorrected data.

ERIC-PCR fingerprinting with the BioNumerics fingerprint data software package (Applied Maths, Sint-Martens-Latem, Belgium)

Normalization steps were included in the analysis of DNA polymorphism patterns produced by ERIC-PCR fingerprinting to ensure an adequate gel-to-gel banding pattern comparison. The process of band scoring was used to identify bands in each lane that combined to make the fingerprint based on the stringency threshold and optimization settings. Dendrograms were generated for the ERIC-PCR gels using the Dice similarity coefficient and the unweighted pair group method using arithmetic averages, with 1% optimization and 1% position tolerance (Bilung *et al.*, 2018). *A. baumannii* strains with a similarity exceeding 90% were considered to be clonally related.

2.2.14.7 Statistical analysis

All frequency data was analyzed by Pearson's chi-squared test and Fisher's exact test . Data were processed and analyzed by using statistical program social science (SPSS22)and the result were expressed percentages (McDonald, 2014).

Chapter Three

Results and Discussion

3. Results and Discussion

3.1 Collection and Isolation of *A. baumanii* Isolates

During the present study period extended from October 2021 to January 2022, a total of (150) clinical specimens were collected from sites suggested to have infection such as burns, wounds infections, respiratory infections ,and urinary infections. All clinical specimens were cultured on different media. All isolates were identified by culturing on blood agar and MacConkey agar and CHROMagar plates and confirm by biochemical test and vitek 2 compact system, The results indicated that 23 (15.3%) of the isolates belonged to *A. baumannii* It was found that 12 (26.6%) isolates obtained from sputum, five isolates (14.2%) detect from burns ,three isolates (7.5%) were detected from wounds, and three isolate (10%) isolated from urine , as shown in Table (3-1).

Table (3-1): Distribution of *A. baumanii* According to Clinical Specimen.

Clinical source	Total No.	Number of *A. baumannii* isolate	Percentage %
Sputum	**45**	**12**	**26.6**
Burns	**35**	**5**	**14.2**
Urine	**30**	**3**	**10**
Wounds	**40**	**3**	**7.5**
Total	**150**	**23**	**15.3**

These results indicate that *A. baumannii* was highly isolated from respiratory infection (26.6%) follow by burns samples and Urine.

Rajkumari *et al.* (2020) found that from 138 (11.49%) isolates *Acinetobacter* were obtained from various specimens. The isolation rate of *Acinetobacter spp* was highest number from sputum sample 44(31.88%) followed by endotracheal tip/aspirate (16.66%), pus (15.21%), tips (12.31%) and urine (10.14%).

Additionally Kaur *et al.* (2016) found that a total of 48 *A. baumanii* isolates were obtain in which (33%) detect from respiratory samples-tracheal aspirate, Endotracheal secretions and sputum (68.7%) followed by pus (12.5%), blood (8.3%), Intercostal drain tube and CSF (4.2%), urine (2.08%), predominance of *A. baumanii* in respiratory sample and it agree with the result obtain in this study were the high isolation detect in respiratory system.

Alatrouny *et al.* (2013) found that at a total of the 55 positive samples , 23 (41.82 %) were detect from sputum, followed by tracheal secretions 11 (20 %) , central venous CV line 8 (14.55 %) , wound 8 (14.55 %) , urine 3 (5.45 %) and blood 2 (3.63%).

The respiratory tract has already been confirmed to be the most common isolation site of *A. baumanii* , also various risk factors noticed have been significantly associated with infection caused by *A. baumanii* like mechanical ventilation ,indwelling catheters and admission of the ICU which increased the percentage of isolated (Lukovic *et al.*, 2020).

Higher isolation rate from respiratory samples probably due to the fact that most patients either had prior respiratory problems or were in ventilators or because of low immunity or severe illness gave the best of opportunity of *Acinetobacter* commensal of upper respiratory tract to become a pathogen or from contaminated hands and objects of the health care workers.

As a consequence of the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) pandemic and the associated disease (COVID-19), patients undergoing mechanical ventilation have soared; these patients are particularly at risk for nosocomial Acinetobacter spp. infections, due to the introduction of assisted ventilation and the preceding viral infection, which predisposes patients to bacterial superinfections. Rapid increases are expected in the resistance rates of Acinetobacter spp. and other bacterial pathogens, associated with the extensive and prophylactic use of antimicrobials in relation with COVID-19 (Rangel *et al.*, 2021) .

But this result is invariance with other studies as by Lahiri *et al.* (2004) and Dimple *et al.* (2016) as they found the highest isolates from tips (43.4%), and Oberoi *et al.* (2009) found the highest isolates detect from pus samples (86.2%).

Other results are produced by Raut *et al.* (2020) found that the high isolation rate of *A. baumanii* from Wound Swab (33.3%), followed by sputum (21.1%) and urine culture (14.9%) .

Ayenew *et al.* (2021) show that a total of 102 *Acinetobacter* strains were isolated from various clinical specimens the major source of the isolates Pus/wound (33.3%), followed by blood (23.5%), urine (15.6%), body fluid (11.7%), ear (4.9%), cerebrospinal fluid (3.9%), tracheal aspirate (1.9%), sputum (0.9%) and throat (0.9%).

The difference in the isolate number sample might be due to size, sources, types of samples, antibiotic usages, and infection control practice in the hospital, which can affect the isolation rate of bacteria from different clinical specimens .

The frequencies of *Acinetobacter* from clinical samples vary by hospital, patient population, exposure to antibiotics, types of patient and changes over time

3.2 Identification of *A. baumanii*

Overall, all the *A. baumannii* isolates were phenotypically identified on both Blood and MacConkey agar with some different characteristics observed as show in figure (3-1 A and B). The morphology of colonies has been seen on the blood agar within 24 h at 37°C. The color growth was white to gray and tiny circular shapes with no hemolysis noticed on colonies and surrounding area due to having no enzyme to hemolyze blood. While, the colonies properties which were appeared on the MacConkey agar at the same lab conditions can be discriminated with mucoid, faint pink, non-fermented lactose sugar, rounded and tiny shapes (Sehree *et al.*, 2021).

Also, a specific and rapid isolation of *A. baumannii* was conducted based on CHROMagar (HiCrome TM) with adding Multidrug resistance selective supplement (FD271). All isolates of growth including (MDR) appeared within (24 to 48 h) at 37°C, as light purple color with halos rounded the colonies as show in figure (3-1c). The positive control of *A. baumannii* (ATCC, 19606) was cultured and the results compared with all isolates to give 100% consistency (Sehree *et al.*, 2021).

The isolates taken from MacConkey agar plate and stained by Gram stain appear under light microscope as a pink coccobacillus wich referred Gram –ve bacteria and arranged in diplococcic. In biochemicals test, all *A. baumannii* isolates showed negative results for oxidase test and motility test, whereas a positive results for catalase.

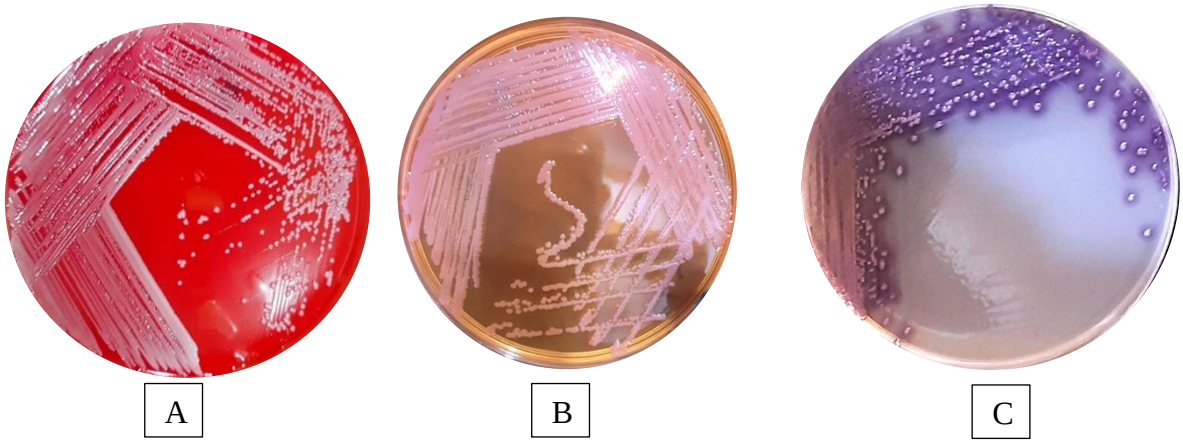

Figure 3-1: Phenotypic characteristic of *Acinetobacter baumannii* colonies, A: on blood agar, B: on MacConkey agar and C: on selective medium CHROMagar™.

3.3 Identification of *A. baumanii* by Vitek - 2 compact system

The clinical isolates identified as *A. baumannii* using VITEK 2 system used the identification card for Gram negative strains (ID-GNB). The obtained result shows an interesting percentage of accuracy (99%) (Appendix).

These results were identical to the findings of Jassim *et al.* (2016). The accurate and rapid identification of *A. baumannii* was critical for the appropriate infection control in hospital settings. Up to date, the most common and widespread detection methods include characterization via a phenotypic system and commercial phenotypic methods (e.g.,the Vitek-2 compact system, Biomerieux). Also , in a study by Almaghrabi *et al.* (2018) who used vitek to successfully identify most *Acinetobacter* species.

3.4 Antibiotic Susceptibility profile of *A. baumanii* by disc diffusion method

All isolates were tested for susceptibility to (10) antibiotics by

using disc diffusion method on Mueller Hinton agar for testing their susceptibility and to identify the most effective one against *A. baumannii.* However, the overwhelmed *A. baumannii* isolates that recovered from clinical specimens were highly resistant to most antibiotics that used in the present study. The antimicrobial susceptibility profile in this study shows the highest resistance percentage 100% were to Cefepime, followed by 91% resistant rate to Trimethoprim- Sulfamethoxazole, 80% resistant rate to Gentamicinand Amikacin, 77% resistant rate to Ciproloxacin, Ceftazidimeand Levofloxacin, 73% and 70% resistant rate to Amoxicillin-clavulanica acid and Meropenem respectively, while low resistance percent 50% was to Tetracycline, the rate of resistance shown in figure 3-2 . This can be attributed to the fact that antibiotics may have revolutionized the treatment of common bacterial infections (WHO, 2014)

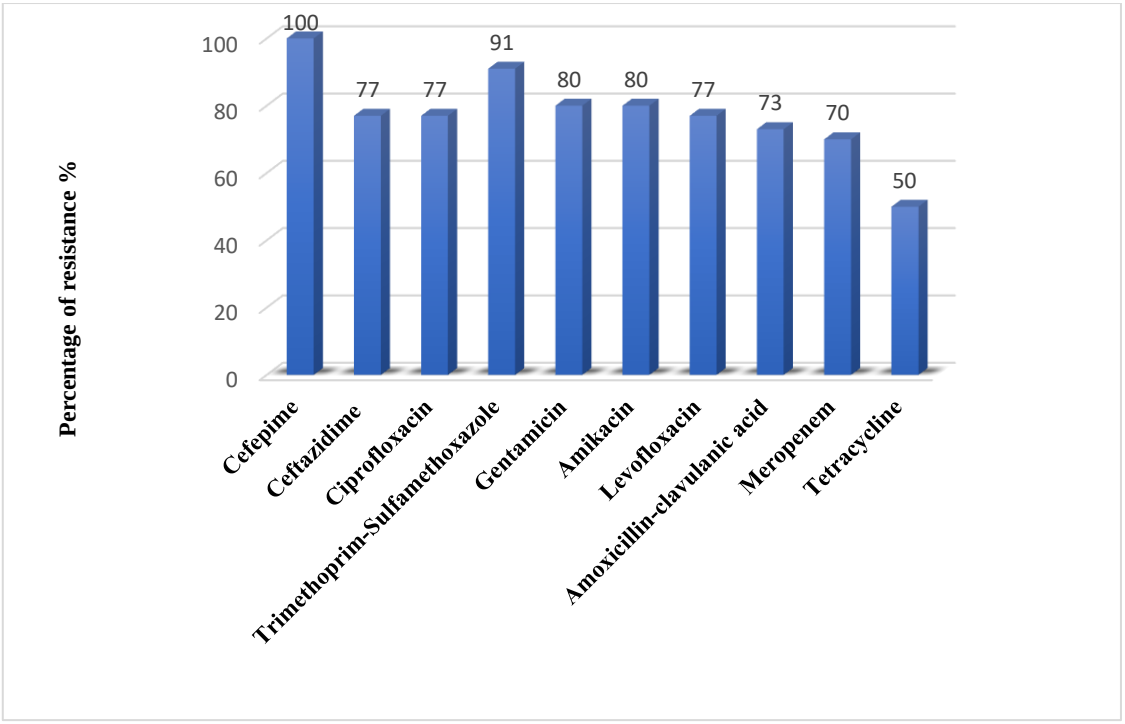

Figure 3.2: Percentage of resistance's isolates *A. baumannii* to different antibiotics

The antimicrobial susceptibility profile revealed a high resistance of *A. baumannii* to Cefepime (100 %), Ciprofloxacin (77%), Ceftazidime (77%), the result was agreement to Alatrouny *et al.* (2020) which found there was a high resistance rate for cefepime (93.3%), ceftazidime (100%),and ciprofloxacin (74.4%). Also, agreements with Raheem and Al-Hasnawy (2020) who found that (100%) resistant to Cefepime and ceftazidime (91.3%) and with Özçelik *et al.* (2020), which was found high rates of resistance to most antibiotics, and he found that (100%) resistant to cefepime, ceftazidime and ciprofloxacin.

The high resistance rate to β-lactam due to high production of β-lactamase which will give longer validity and surviving to this bacterium, because the hydrolysis of β-lactams takes place before the drug can bind to PBPs in the cell membrane (Yahav *et al.*, 2020).

Also, the present study found that the resistant rate of Trimethoprim-Sulfamethoxazole Ibrahim *et al.* (2021) (91%), this result agrees with Smiline Girija (2019) and with Sehree *et al.* (2022) whose showed that the resistant rate is (91.78%) and (91.2%) respectively.

The results of the present study found that (80%) are resistant to Gentamicin, Amikacin , this result was agreed with the results that found in (Saudi Arabia) by Ibrahim *et al.* (2021), *A. baumannii* revealed the highest resistance rates for gentamicin (88%) and amikacin (86%) , And with Özçelik *et al.* (2020) found that of *A. baumannii* isolate were resistant(68%) for amikacin, (78%) for gentamicin. Also, agreements with Sehree *et al.*, (2022) who found that resistance rates for Gentamycin was (93.6%), and Amikacin (87.6%).

The production of 16S RNA ribosomal methyltransferase, especially ArmA, is a mechanism of resistance to aminoglycosides, *A. baumannii* strains that produce ArmA are highly resistant to gentamicin, amikacin, and tobramycin (da Paz Pereira *et al.*, 2020).

The results in this study found resistance rate to Levofloxacin was (77%) this result was agreement to the result detect by Shen *et al.* (2016) who showed that (76.24%) isolates were resistant to levofloxacin.

Moreover, the results in this study refer resistance rate to Amoxicillin-clavulanic acid (73%) this result was agreement to the result detect by Nakbubpa *et al.* (2021) show high resistant percentage to amoxicillin-clavulanic acid (84.62%).

(Penicillin-binding protein) PBP2 has been shown to have the highest affinity for penicillin and beta lactamase inhibitors. *A. baumannii*'s PBP2 to initiate an effect against the microorganism , The resistance of *A. baumannii* is associated with a reduced expression of PBP2 and β-lactamase inhibitors, such as clavulanic acid (Vázquez-López *et al.*, 2020)

The result of resistance level for Meropenem in this study was (70%) which agree with Sehree *et al.* (2021) who found that the resistance (85.3%) for Meropenem, and agreement with Raheem and Al-Hasnawy, (2020) showed high rates of resistance to most antibiotics used ,like resistant to Meropenem (62.2%).

Resistance occurs when the organism including an altered PBP that is encoded by the genes in *A. baumannii* can be constitutive or acquired by means of integrons, transposons, and plasmids. They encode both the enzymes that modify the antibiotic molecules and the modifications of the antibiotic target sites. These genes also code for the efflux pump proteins and porin channels of the cell membrane, both systems related to the decrease in the intracytoplasmic concentration of the antibiotic(Bello and Dingle, 2018) .

The results in this study show resistance rate to Tetracycline (50%) this result was identical to tetracyclines resistant percentage (50–60%) showed by Mahich *et al.* (2021),while (31.7%) resistant percentage

foundin study done by (Sehree *et al.*, 2021).

Resistance against tetracyclines is mediated by various mechanisms, including active efflux of the antimicrobial mediated by resistance proteins in the bacterial cytoplasmic membrane and inhibition of ribosomal and tetracycline binding (Zhang *et al.*, 2021).

The increasing rates of antibiotic resistance can be attributed to multiple causes. These is a direct relationship between the emergence of resistant strain and the magnitude of antibiotic consumption, resistance mechanisms can be transition from one bacterium to anther by either longitudinally ,when inherited from relatives or horizontally by means of plasmids, the latter may result in the transference of resistance among difference species . Also, massive agricultural use of antibiotics, also the availability and low cost of antimicrobials has led to considerable redaction of investment into new alternative by the pharmaceutical industry (Vázquez-López *et al.*, 2020).

On the report of the World Health Organization (WHO) published in 2017, there was an urgent need for new antibiotics for 6 pathogens called the ESKAPE (ESKAPE is the acronym for the group of bacteria that include *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa, and Enterobacter* spp.) (Mulani *et al.*, 2019).

Acinetobacter has been considered a reservoir of antibiotic-resistant genes in a hospital environment; this is confirmed by results which showed high resistance rates to most antibiotics used. (Kong *et al.*, 2011).

The high resistance rates recorded in the study are likely to be associated with a wide range of empirical and therapeutic use of antibiotics at hospitals. The employed selective pressure by this results in MDR strains emerging, which in turn may have led to the genes encoding resistance mechanisms (Mohammed *et al.*, 2022).

3.5 Detection of biofilm formation by *A. baumanii* using tissue culture plate method (TCP)

A total of (23) isolates of *A. baumannii* were tested for their ability to produce biofilm. And the result show that all the isolates 23 (100%) were strong biofilm formation as shown in table 3-2.

Table 3-2: Result of Tissue culture plate method (TCP)

Isolates (No.)	Biofilm formation No.(%)		
	Strong	Moderate	None/Weak
Acinetobacter baumannii (23)	23 (100%)	0 (0%)	0 (0%)

Tissue culture plate (TCP) method defined by Christensen *et al.* as gold standard assay, was used for identification of biofilm formation (TCP) method is a more quantitative and reliable method for the detection of biofilm-forming microorganisms as compared to other methods. In this method, biofilm formation was considered positive when a visible film lined the wall and bottom of the tube biofilm are formed and gave a high optical density value compared to the control Differentiation of *A. baumannii* isolates as biofilm producers or non-biofilm producers was done by using 96 wells micro titer plate and ELISA reader according to mean of OD value at 570nm. The results were interpreted as none, moderate and high biofilm producer when the mean of OD value is (< 0.120 Non/weak _ 0.120- 0.240 Moderate _ > 0.240 Strong) (Sultan and Nabiel, 2019).

Pompilio *et al.* (2021) and Sarshar *et al.* (2021) found that the majority of *Acinetobacter* isolates (62.14% overall) were strong biofilm producers, both in the MDR and non-MDR groups; there tests conducted did not verify a significant difference in biofilm formation based on the susceptibilities of the relevant isolates to the tested antimicrobials. Based

on the reports from the recent literature, the rate of biofilm formation in *A. baumannii* is around 75–100%, which is similar to rates observed in nonfermenting Gram -negative bacilli, and significantly higher than those of members of the *Enterobacterales* and Gram-positive bacteria (0–40%).

Al-Shamiri *et al.* (2021) reported that a among the 70 tested isolates,51.4% were strong biofilm formers, 41.4% were moderate biofilm formers, and 4.3% were weak biofilm formers , only 2.9% were unable to form biofilms. and all these *A. baumannii* isolates produced biofilm were resistant to antibiotic.

Zeighami *et al.* (2019) reported that all isolates were able to produce biofilm and 58% of isolates showed strong ability to biofilm formation. All strong biofilm forming *A. baumannii* isolates were XDR. All *A. baumannii* isolates carried at least one biofilm related gene. The most prevalent gene was *csuE* (100%), followed by *pgaB* (98%), *epsA* and *ptk* (95%), *bfmS* (92%) and *ompA* (81%). 98% of isolates carried more than 4 biofilm related genes, simultaneously.

Mangas *et al.* (2019) compared nearly 2000 *A. baumannii* genomes, they observed that strains carrying CRISPR systems were enriched for biofilm-associated genes (>70 vs <2% non-CRISPR strains), suggesting a link between CRISPR immunity and biofilm formation .

Previous research has shown that *Cas*3 endonuclease is involved in the control of biofilm formation in both Gram -positive and Gram -negative bacteria (Colquhoun and Rather, 2020).

Several mechanisms considered key factors in the high resistance of biofilms have been explored: limited diffusion, enzyme-caused neutralizations, heterogeneous function, slow growth rate, biofilm phenotype adaptive mechanisms (Yang *et al.*, 2019).

A. baumannii has the ability to form biofilm on a wide range of surface including abiotic surface as well as host epithelial cell many

virulence factors have been implicated in bacterial cell adherence however the plasticity observed in *A. baumannii* genomes leads to significant strain-specific variations in biofilm formation and the presence of known biofilm-associated genes in *A. baumannii* (Colquhoun *et al.*, 2020).

3.6 Effect of $AgTIO_2$ and SWCN-OH Nanoparticles on *A. baumannii* growth

In this study the effect of $AgTIO_2$ and SWCN-OH was examined against *A. baumannii* isolates growth by making five double serial dilutions at (1/2, 1/4, 1/8, 1/16 and 1/32) form stock solutions (concentration 1000 ug/ml); then after incubation for 24 and 48 hours the bacterial growth had been monitored and checked with spectrophotometer.

Results showed that there was a significant ($p \leq 0.05$) decrease in growth of bacteria after 24hr and 48hr of incubation with different concentrations of $AgTIO_2$ and SWCN-OH nanoparticles especially with the first and second dilution, as shown in figure (3-3) (3-4). Moreover, the result indicates that 48hr more effective in growth inhibition than 24hr.

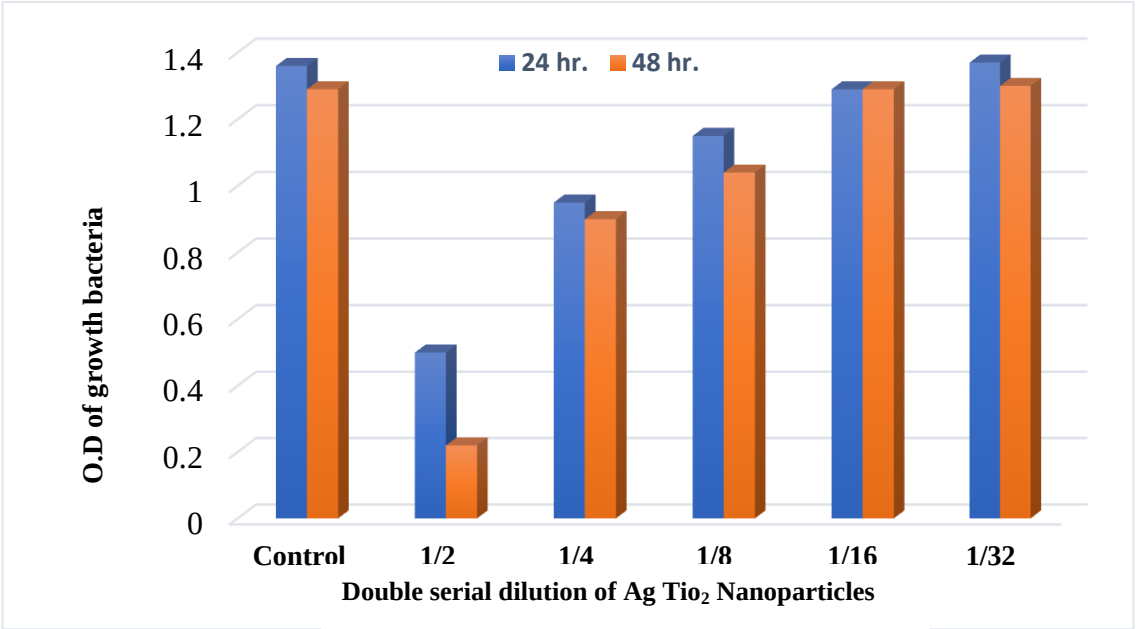

Figure 3 -3: Effect of double serial dilution of Ag Tio_2 Nanoparticles on *A. baumannii* growth ($p \leq 0.05$)

The highest concentration of Ag Tio_2 after diluted for (1/2) made the highest inhibition effect for bacterial growth and this inhibition minimized the bacterial growth from (1.36) to (0.5) after 24hours of incubation while after 48 hours of incubation bacterial growth minimized from 1.29 to 0.22. While the lowest concentration of Ag Tio_2 that made by diluted the nanoparticles for (1/32) showed the lowest inhibition effect on *A. baumannii* growth after 24 and 48 hours of incubation.

As an antibacterial agent, silver-titanium dioxide nanocomposite materials are widely known for their anti-pathogenic ability; studies have reported efficacy against various strains of Gram-positive bacteria (*B. subtilis, S. aureus*, MRSA), Gram-negative bacteria (*E. coli, K. pneumonia, P. aeruginosa*) and fungi (C. albicans) *(*Muflikhun *et al.*, 2019*).*

Ag-TiO_2 nanoparticles has been discussed to be a solution to antibiotics resistance problem. It also can destroy microbes and bioaerosols (dust mites, mold spores) by disintegrating their DNA. Silver nanoparticles may directly attach to and penetrate the cell membrane to kill spores, although penetration of silver nanoparticles into microbial cell membranes is not completely understood. The use of the low-cost photocatalyst and the possibility of its activating with solar light offer economically reasonable and environmentally friendly solutions to the disinfection process, development of self-cleaning materials and the protection of technical materials from biodeteriorating microorganism (Ahmad Barudin *et al.*, 2013).

Several mechanisms have been reported for the antimicrobial property of silver doped TiO_2 nanoparticles ,The first step is that the adhesion of nanoparticles to the cell surface rated by the TiO_2 photocatalyst attacks the outer membrane. The second step is the effective uptake of the silver ions into the cytoplasmic membrane, degrade lipoolysaccharide molecules, accumulate inside the membrane and cause membrane permeability ,Silver particles penetrating inside bacterial cell, thus damage

the DNA Dissolution of silver releases antimicrobial Ag ions .In this case, the TiO2 photocatalytic reaction assists the intrusion of the silver ions into the cell (Ahmad Barudin *et al.*, 2013).

The main mechanism of TiO_2 NPs toxicity is potentially associated with metal oxides carries the positive charge even though the microorganisms bear negative charges; this results in electromagnetic interaction between microorganisms and metal oxides leading to oxidation and finally death of microorganisms. Bactericidal action of TiO_2 nanoparticles on bacteria is of extreme importance due to the ability of pathogenic bacteria to join the food chain of the ecosystem . The antibacterial activity of TiO_2NPs was due to the capability of TiO_2 particles to cause free hydroxyl radicals (OH^-) (Hamza and Yaaqoob, 2020).

Similarly, for SWCN-OH the highest concentration at diluted (1/2) made the highest inhibition effect for bacterial growth and this inhibition minimized the bacterial growth from (1.36) to (0.1) after 24hours of incubation while after 48 hours of incubation bacterial growth minimized from 1.29 to 0.1. The lowest concentration of SWCN-OH that made by diluted the nanoparticles for (1/32) showed the lowest inhibition effect on *A. baumannii* growth after 24 and 48 hours of incubation as shown in figure 3-4.

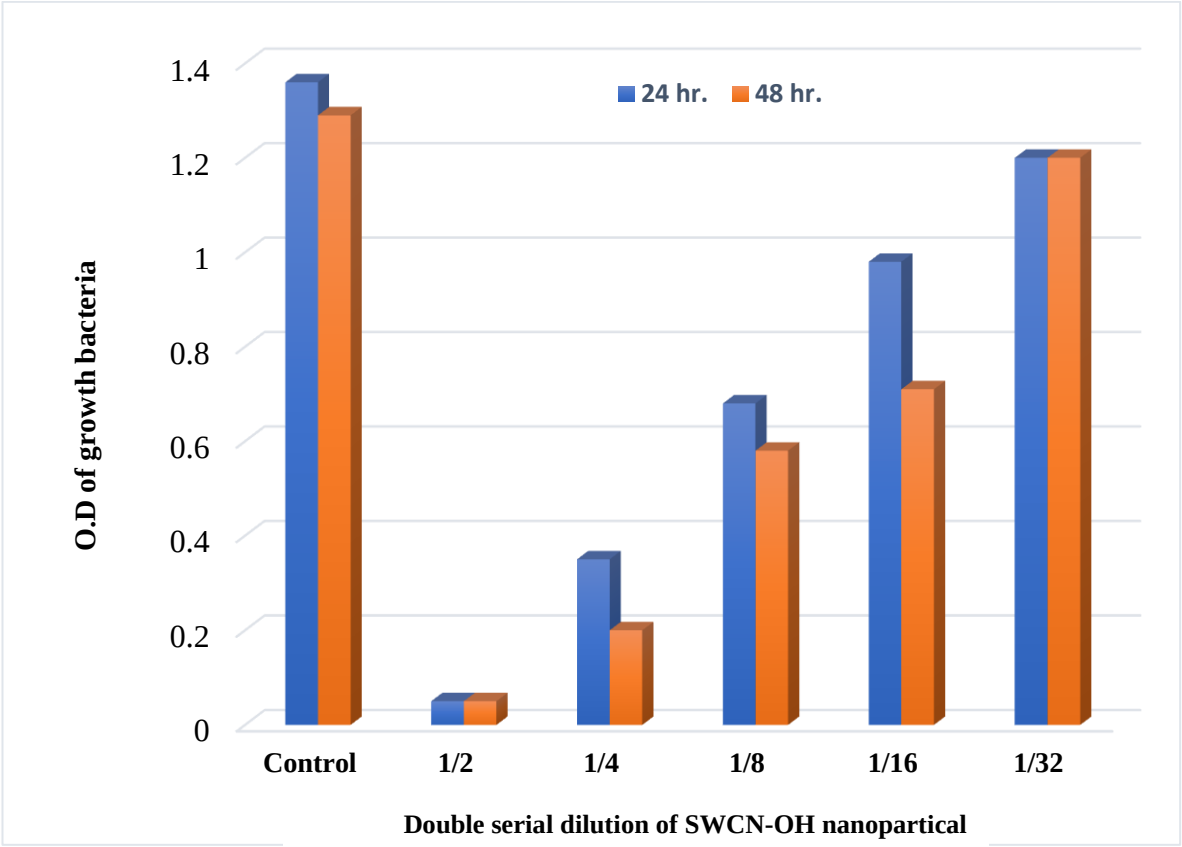

Figure 3-4: Effect of double serial dilution of Single Walled Carbon Nanotubes (SWCN-OH) on *A. baumannii* growth

Dong *et al.* (2012) demonstrated that SWCNTs having surface groups of –OH and –COOH exhibited extremely strong antimicrobial activity to both Gram-positive and Gram-negative bacterial cells.

carbon nanotubes have been exploiting numerous biomedical applications such as drug carrier ,biosensor and tumor hyperthermia Despite the widely application of carbon nanotubes, a number of studies have been reported on the potential toxic effects of the nanomaterials to lungs and human cells. On the other hand, the toxic carbon nanotubes were used to kill undesirable microorganisms (Li *et al.*, 2014).

Carbon nanotubes showed good bactericidal and bacteriostatic activity when bacteria get thoroughly exposed to them, and the antibacterial

activity were closely related with its size and specific surface area, for this reason, single-walled carbon nanotubes (SWCNTs) displayed stronger antibacterial activity than multi-walled carbon nanotubes (MWCNTs) (Li *et al.*, 2014).

The decrease in the bacteria growth may be due to the three-step SWNT antimicrobial mechanism involving (i) initial SWNT−bacteria contact, (ii) perturbation of the cell membrane, and (iii) electronic structure-dependent bacterial oxidation (Vecitis *et al.*, 2010).

3.7 Effect of $AgTIO_2$ and SWCN-OH on the biofilm formation by *A. baumanii*

In this study the effects of $AgTIO_2$ and SWCN-OH were examined against biofilm formation in *A. baumanii* isolates by making four double serial dilutions from stock concentrations as (1/2, 1/4, 1/8, and 1/16) and those concentrations were (1000 μg /ml). The results showed that there was a highly significant ($p \leq 0.05$) decrease in biofilm production in bacteria after exposure to the different concentrations of $AgTIO_2$ and SWCN-OH nanoparticles after 24hr, the ability of *A. baumanii* to form biofilm had been monitored and checked with spectrophotometer and the first diluent is the best one. as expressed in figure 3-5, 3-6.

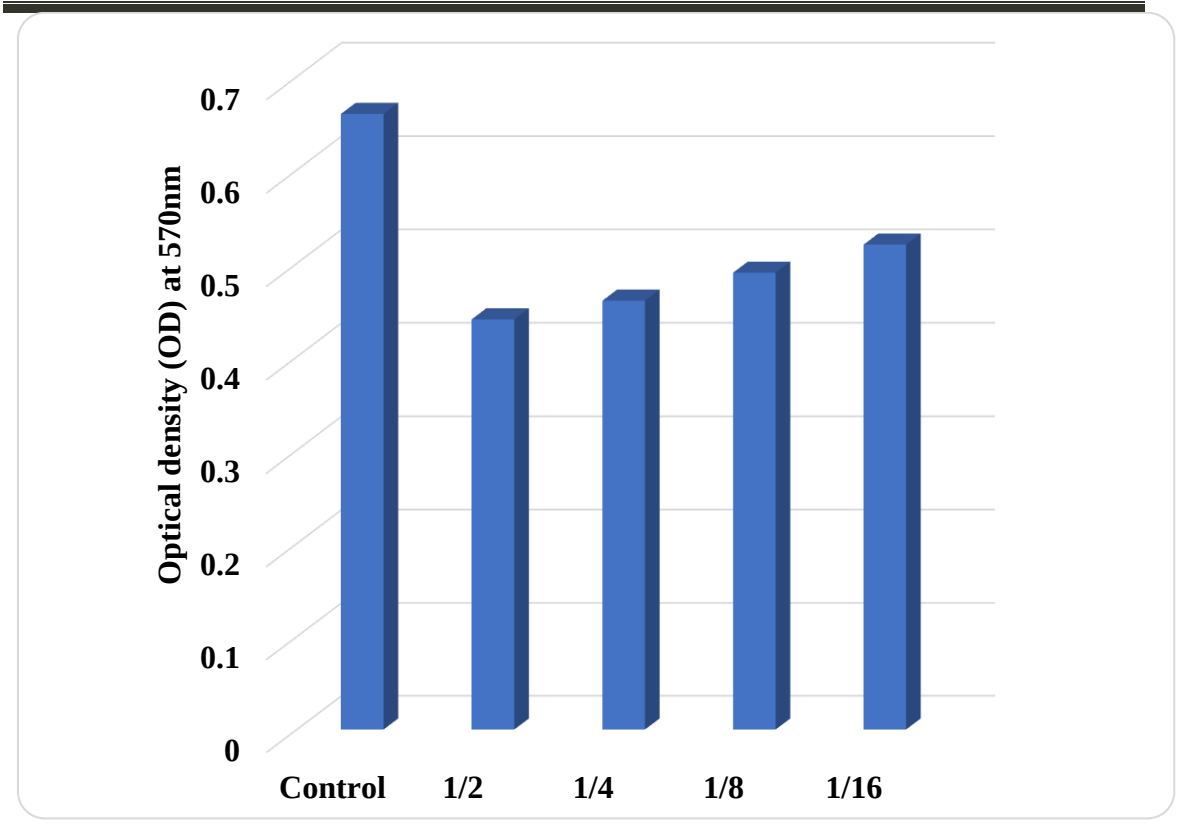

Figure 3-5: *A. baumanii* biofilm inhibition by double serial dilution of $AgTIO_2$ Nanoparticles

Alavi *et al.* (2018) reported that the biofilm formation in *E. coli*, *S. aureus*, and *P. aeruginosa* higher sensitivity to Ag–TiO_2 in 78.05%, 82.15% and 86.52% respectively at value of 100 µg/mL.,the antibiofilm ability of NPs can be resulted from disrupting of biofilm structure and penetration of metal ions into under layers of bacterial colony which is not accessible for common antibiotics .

Biofilms are difficult to eradicate with conventional disinfectants, the activity of disinfectants such as metal oxide nanoparticles (NPs) is increasing. In the last decade, many studies describing the photocatalytic inactivation of bacteria using doped and undoped TiO_2 coated on different substrates have been reported, including silver doped TiO_2.

Natarajan (2015) showed that the biofilm was highly inhibited by Ag-TiO2 ,The Ag-TiO2 composite (1 wt%) showed 77%biofilm inhibition in *E. coli* while in *S. aureus* biofilm showed 67% ihibition after 18 h of incubation, The higher activity of these composites against *E. coli* a Gram-

negative bacterium is attributed to its thinner peptidoglycan cell wall compared to *S. aureus* a Gram-positive bacterium. antibiofilm activity of composite is directly proportional to Ag-TiO_2 loading. Exposure of the composite with 1.5 wt% Ag-TiO_2 for 24 h. resulted complete inhibition of biofilm in case of both *E. coli* and *S. aureus.*

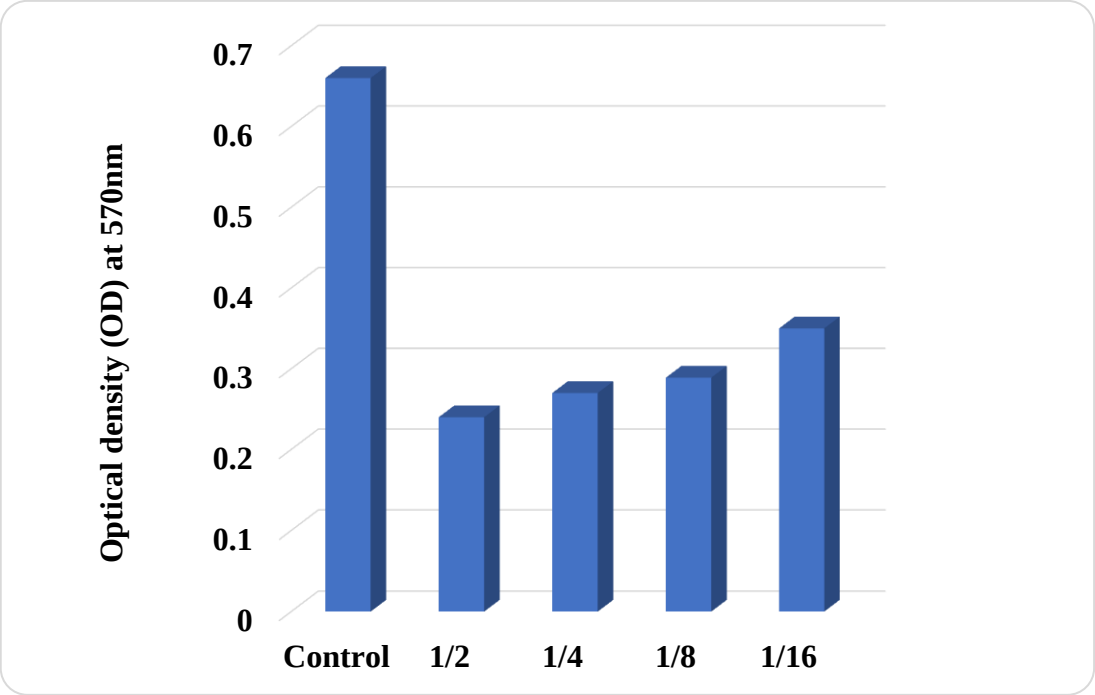

Figure 3-6: *A. baumanii* biofilm inhibition by double serial dilution of SWCN-OH Nanoparticles

The increasing number of biofilm-related infections has motivated the development of effective strategies to prevent microbial adhesion and biofilm formation on medical surfaces (Chine *et al.*, 2016).

CNTs are attractive materials with proven antimicrobial and anti-adhesive activities against a broad range of Gram-positive and -negative bacteria, including drug-resistant microorganisms and fungi (Teixeira-Santos *et al.*, 2021).

Microorganisms respond to the CNTs. Numerous toxicity mechanisms have been proposed for CNT, comprising, disruption/ penetration of the cell envelope, oxidation of cell components, interruption of trans membrane electron transfer, and formation of secondary products

such as reactive oxygen species (ROS) or dissolved heavy metal ions. Toxicity of a CNT is dependent on its along with its geometry and surface functionalization. Whereas CNTs without functionalization appeared severely toxic to human or animal cells lines at moderate dosage (Khalid *et al.*, 2016).

Al-Jumaili *et al.* (2017) studied the lethal effects of CNTs on biofilm formation to evaluate their potential to impede microorganism attachment and proliferation at different stages of bacterial colonization. Biofilm structure provides significant protection for bacterial cells and renders them highly resistance to detachment by physical forces and harmful nanoparticles. CNTs have shown strong bactericidal activities towards cells in biofilms, . It has been reported that microscopic examinations to the bottom layer of the biofilms of *E. coli* and *B. subtilis* in direct contact with coatings containing SWCNT showed that ~80–90% of the microbial cells were dead . Yet, the interaction of CNTs with biofilm is highly dependent on the stage of biofilm formation, where the efficacy of CNTs is more pronounced at the early steps of biofilm formation . As soon as microorganisms become protected within the structure of the mature biofilm, they are less susceptible to the influence of CNTs than bacteria in other biofilm phases.

The anti-adhesive effect can be caused by the mobility of CNTs, which create an unstable substrate, and thereby affecting appropriate microbial adhesion. CNTs inhibit microbial biofilms in a concentration dependent mode: 50 μg / mL−1 SWCNTs reduction the biofilm by 81.19%, and ≥200 μg / mL−1 SWCNTs totally inhibit the biofilm . However, more in-depth understanding of how CNTs interact with biofilms is needed to engineer appropriate nanomaterial agents to effectively disturb microorganisms at any growth phase or biofilm stage (Malek *et al.*, 2016).

3.8 Molecular Detection of *cas* genes in *A. baumanii*

The CRISPR/Cas systems are one of the phage immunity systems that are present in bacteria and enabling the organisms to respond and eliminate invading genetic material. Across the 20 strains In this study, amplicons of *cas*1 (506 bp) , *cas*2 (196 bp) and *cas3* (850 bp) genes were present in 5(25)% , 3(15)% , 9(45)% isolates respectively. Amplicons for *cas* 5, *cas* 6 and *cas* 9 were 611bp, 432bp and 247bp respectively.

The *cas* 5 gene was found in 3(15)% isolates, while *cas* 6 was detected in 7(35)% isolates, the *cas*9 gene was found 6(30)% as shown in table(3- 3) and figure (3-7)(3-8).

Table 3-3: Identification of *cas* genes of *A. baumanii* in all studied samples

Results	***cas1*** **N (%)**	***cas2*** **N (%)**	***cas3*** **N (%)**	***cas5*** **N (%)**	***cas6*** **N (%)**	***cas9*** **N (%)**	**P value**
Positive	5(25)	3(15)	9(45)	3(15)	7(35)	6(30)	<0.001
Negative	15(75)	17(85)	11(55)	17(85)	13(65)	14(70)	
Total	20	20	20	20	20	20	

* represent a significant difference at p<0.001.

Table 3-4 : Identification of *Cas* genes of *A. baumannii* in patients with different sample sources.

Sample Sources	*cas1* N (%)	*cas2* N (%)	*cas3* N (%)	*cas5* N (%)	*cas6* N (%)	*cas9* N (%)
Urine	2/3 (66.6)	1/3 (33.3)	3/3 (100)	1/3 (33.3)	2/3 (66.6)	1/3 (33.3)
Sputum	1/9 (11.1)	1/9 (11.1)	2/9 (22.2)	1/9 (11.1)	3/9 (33.3)	3/9 (33.3)
Burns	1/5 (20)	1/5 (20)	3/5 (60)	1/5 (20)	2/5 (40)	1/5 (20)
Wound	1/3 (33.3)	0 (0)	1/3 (33.3)	0 (0)	0 (0)	1/3 (33.3)
Total	5/20	3/20	9/20	3/20	7/20	6/20

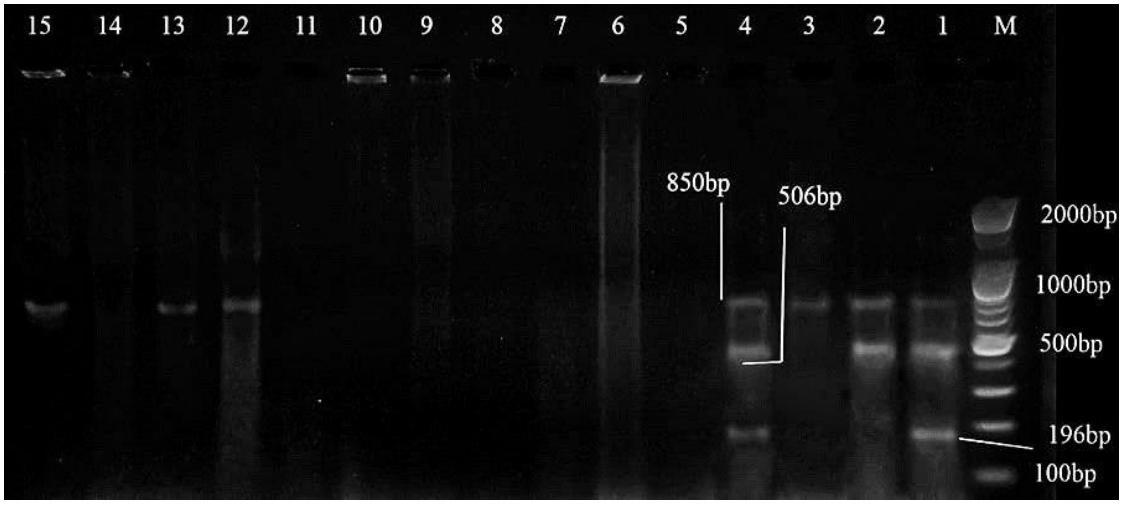

Fig. 3-7 : Agarose gel electrophoresis of Multiplex-PCR products obtained by using *cas1*, *cas2* and *cas3*-specific primers. the lanes 1-4, 12, 13and 15 represent the identified *cas1* or *cas2* or *cas3* gene products with 506bp for cas1, 196bp for *cas2*, 850bp for *cas3*, Lane M represent 100bp DNA ladder ,the electrophoresis was performed at 70 volt for 1 hr.

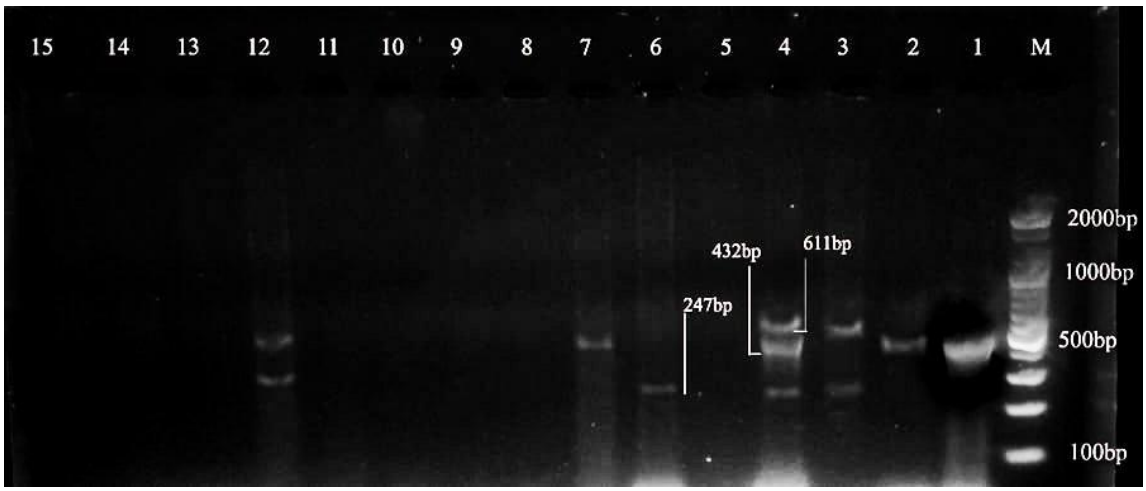

Fig. 3-8 : Agarose gel electrophoresis of Multiplex-PCR products obtained by using *cas5*, *cas6* and *cas9*-specific primers. the lanes 1-4, 6, 7 and 12 represent the identified *cas*5 or *cas*6 or *cas9* gene products with 611bp for *cas* 5, 432bp for *cas6*, 247bp for *cas9*, Lane M represent 100bp DNA ladder, the electrophoresis was performed at 70 volts for 1 hr.

The CRISPR- CAS system is composed of the *cas* genes organized in operons and the CRISPR arrays, which comprises two structures, the spacer target sequences from the foreign nucleic acid and the repeats, identical sequences interspersed with the spacers (Garrett, 2021).

Leungtongkam *et al.* (2020) who found that the *A. baumannii* contain 32 (13.91%) *cas*1 , 2 (0.87%) *cas2* and 30 (13.04%) of *cas3* isolates. The *cas5* gene was found in 34 (14.78%) isolates, while *cas6* was detected in 7 (3.04%) isolates. The *cas9* gene was not found among any of the *A. baumannii* isolates.

While Lin *et al.*, in (2016) showed that *Klebsiella pneumoniae* isolated from the hospital environment have a decreased level of phage immunity and a reduction in CRISPR-Cas activity compared to strains isolated from outside the hospital environment. PCR with cas-specifc primers showed that 19% of the *A. baumannii* isolates had a CRISPR-Cas system.

Cas1, cas2, and cas3 all belong to type 1 system, this protein found as cascade –like complex which involved in target localization, spacer acquisition and cr RNA processing (Butiuc-Keul *et al.*, 2022).

The *cas*1is the most conserved gene with low resemblance to other genetic components, however the gene is prone to module shuffling ,*cas3*is the signature gene ,despite being a multi domain protein and close resemblance to other genetic components and low degree of conservation , (Makarova *et al.*, 2018) .

CRISPR-cas function in the evolution of prokaryotes and the molecular mechanisms still remain unknown. Several studies have

suggested that CRISPR-cas system also could be involved in biofilm formation, colonization and virulence regulation (Newsom *et al.*, 2021).

A previous study found that the genes involved in biofilm formation appeared almost exclusively in the group enriched in CRISPR systems. The loss of function of proteins involved in CRISPR systems, such as the endonuclease *Cas3*, seems to affect to biofilm formation (Tang *et al.*, 2019), then CRISPR systems could help in the survival of the bacterium on inert surfaces, which is meaningful for the resistance of *A. baumannii* in hospitals (Peleg *et al.*, 2008) .The lack of CRISPR gene is related to species, multidrug resistance and major drug resistance –related gene (Dos Santos *et al.*, 2020).

A previous study demonstrated clear association of MDR genotype/ phenotype of *A. baumannii* with the type of its CRISPR/Cas system. Isolates lacking both CRISPR arrays and active *cas* genes were shown to have much more antibiotic resistance genes than those having only CRISPR arrays or both CRISPR arrays and *cas* genes . This complies with the fact that CRISPR/Cas system represents a so-called prokaryotic "immune system" that can fight not only against phage infections, but also against dissemination of antibiotic resistance genes in *A. baumannii*(Tyumentseva *et al.*, 2021).

The diversity could be due to the fact that they due to the genetic instability were obtained from different sources of sample.

3.9 Molecular Detection of *I-F CRISPR-Cas gene* in *A. baumanii*

Type I CRISPR/Cas systems include seven subtypes, I-A to I-F and I-U. Among those subtypes, the I–F CRISPR–Cas system is one of important protein that aim to programmable transcription activation and have important applications in genome engineering (Liu *et al.*, 2020).

Out of 20 strains in this study, the *I-F CRISPR-Cas* gene detected in 7 strains as (table 3-5 ,fig 3-9) isolated from different sources as following: Urine 3 (100%), Sputum 2 (22.22%), Burns 1 (20 %) and Wound 1 (33.3 %) as shown in table (3-6).

Table 3-5 : Identification of *I-F CRISPR-Cas* gene among all studied *A. baumannii* samples.

Results	N	Percentage	P value
Positive	7	35%	<0.001
Negative	13	65%	
Total	20	100%	

* represent a significant difference at p<0.001

Table 3-6 : Identification of *I-F CRISPR-Cas* gene of *A.baumannii* in patients with different sample sources.

Sample Sources	*I-F CRISPR-Cas* N (%)
Urine	3 /3 (100)
Sputum	2 /9 (22.22)
Burns	1 /5 (20)
Wound	1 /3 (33.3)
Total	7 /20

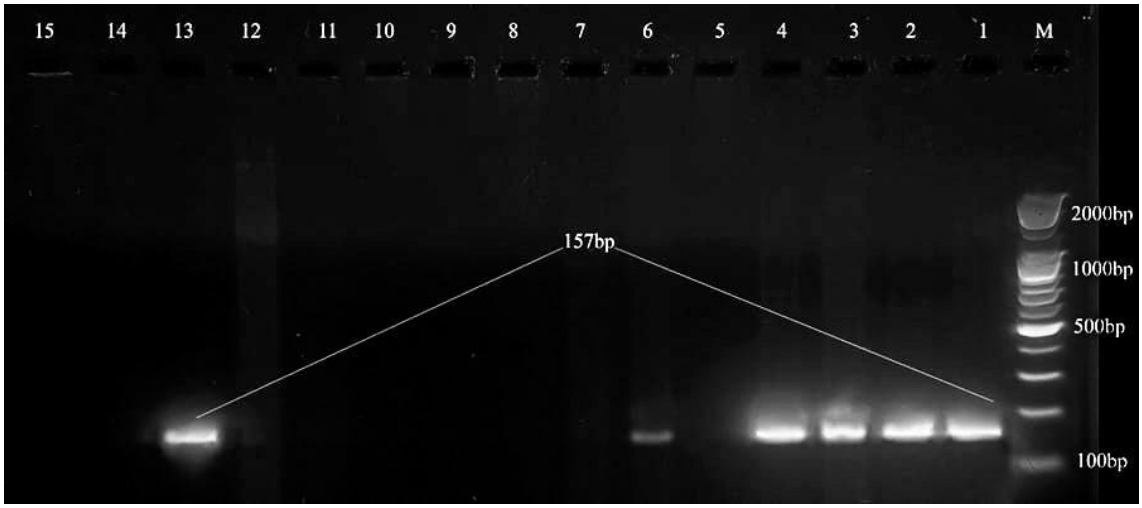

Fig. 3-9 : Agarose gel electrophoresis of Uniplex-PCR products obtained by using A subtype I-F CRISPR-Cas system-specific primers. The lanes 1-4, 6, and 13 represent the identified *I-F CRISPR-Cas* gene products with 157bp. Lane M represents 100bp DNA ladder, the electrophoresis was performed at 70 volt for 1 hr.

In the I-F1 CRISPR/Cas system multiple *cas* proteins and CRISPR RNA(crRNA) from surveillance complex for target recognition (Guo *et al.*, 2022).

TypeF I-F1 CRISPR/Cas system is the closest relative, The Type I-F1 CRISPR/Cas system is characterized by a unique fusion of *Cas2* to *Cas3* (*Cas2*/3), which together with *Cas*1 mediate spacer integration into CRISPR locus, *Cas*2/3 also degrades foreign DNA, which is targeted by the Type I-F1 cascade (i.e., CRISPR-associated complex for antiviral defense) (Tyumentseva *et al.*, 2021).

Karah *et al.* (2015) reported that a vertical transmission of the CRISPR-Cas subtype I-Fb in a global collection of 76 *A. baumannii* isolates with occasional events of horizontal transfer have increased the diversification and facilitated further dissemination of subtype I-Fb. Taken together, these results raised the possibility of horizontal transfer of CRISPR-Cas systems among *K. pneumoniae* isolates. Moreover, CRISPR-based genotyping approach has been expanded to the analysis of

populations and dissemination routes of particular isolates, with the ability to truly assess genetic diversity even within relatively clonal set of bacteria (Barrangou *et al.*, 2016).

Therefore, it is interesting to determine the efficacy of CRISPR typing in epidemiological surveillance and outbreak investigation of *K. pneumoniae* in the future.

Hauck *et al.* (2012) reported approximately 70% (14/20) of the strains with a CRISPR-Cas I-Fb system presented MDR and XDR profiles, which has not been observed in other studies since there is no correlation between the previous profiles.

3.10 Detection of phylogenetic diversity of *A. baumanii* isolated from different samples by (ERIC)-PCR

By using ERIC primer, *A. baumannii* performed different DNA patterns with amplicons that gave a polymorphic band varied in size from (100-more than 2000) bp, the finger printing Patterns of the isolates were shown 16 bands on gel Electrophoresis as shown in figure 3-10

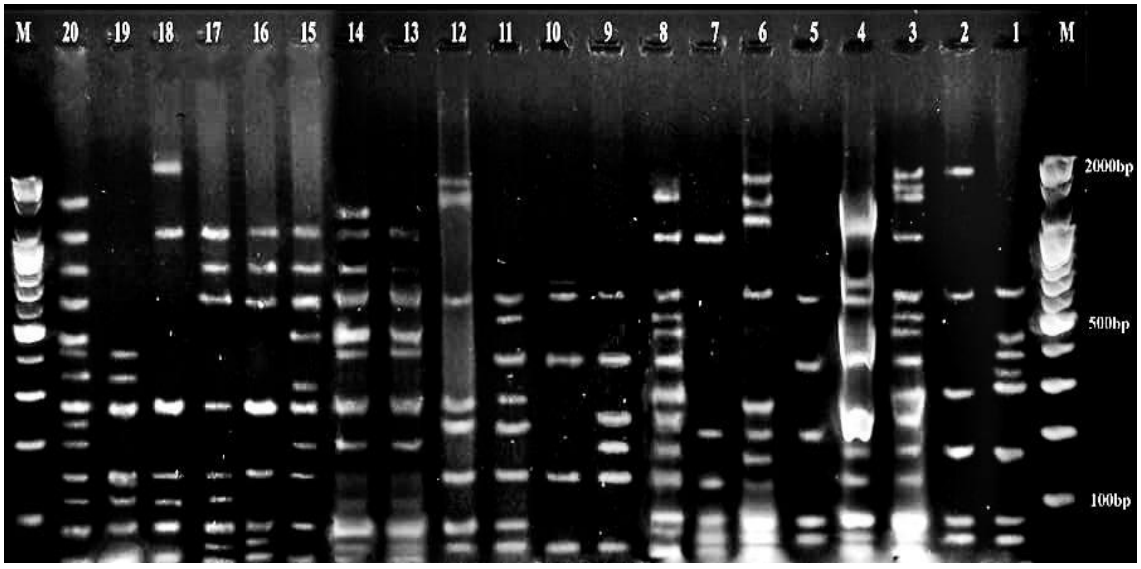

fig 3-10 :Agarose gel electrophoresis of uniplex-PCR products ERIC-PCR digit,the electrophoresis was performed at 70 volt for 1 hr.

ERIC -PCR fingerprinting grouped *A. baumannii* strains isolated from different specimens and from the same period of isolation and location. Among the (20) collected strains of *A. baumannii* the 20 strains were divided in to two clade A,B in which clade A contain two sub cluster , contain 8 isolates and 3sub group and 2 unique as following: first sub cluster belongs to wound and burn sample, the second and third belongs to sputum samples one the other hand, there's a two unique sub clusters both of them belongs to wound isolates and clade B contain 2 sub group including 4 sub clusters as following: first sub cluster belongs to burn isolates the second and third belongs to sputum isolated while the four sub clusters belongs to urine isolates. With regard to the unique sub clusters of B clusters there are four unique sub-clusters. The first and second one belonging to burn, third one belongs to sputum and the fourth belongs to urine **Fig** 3- 11.

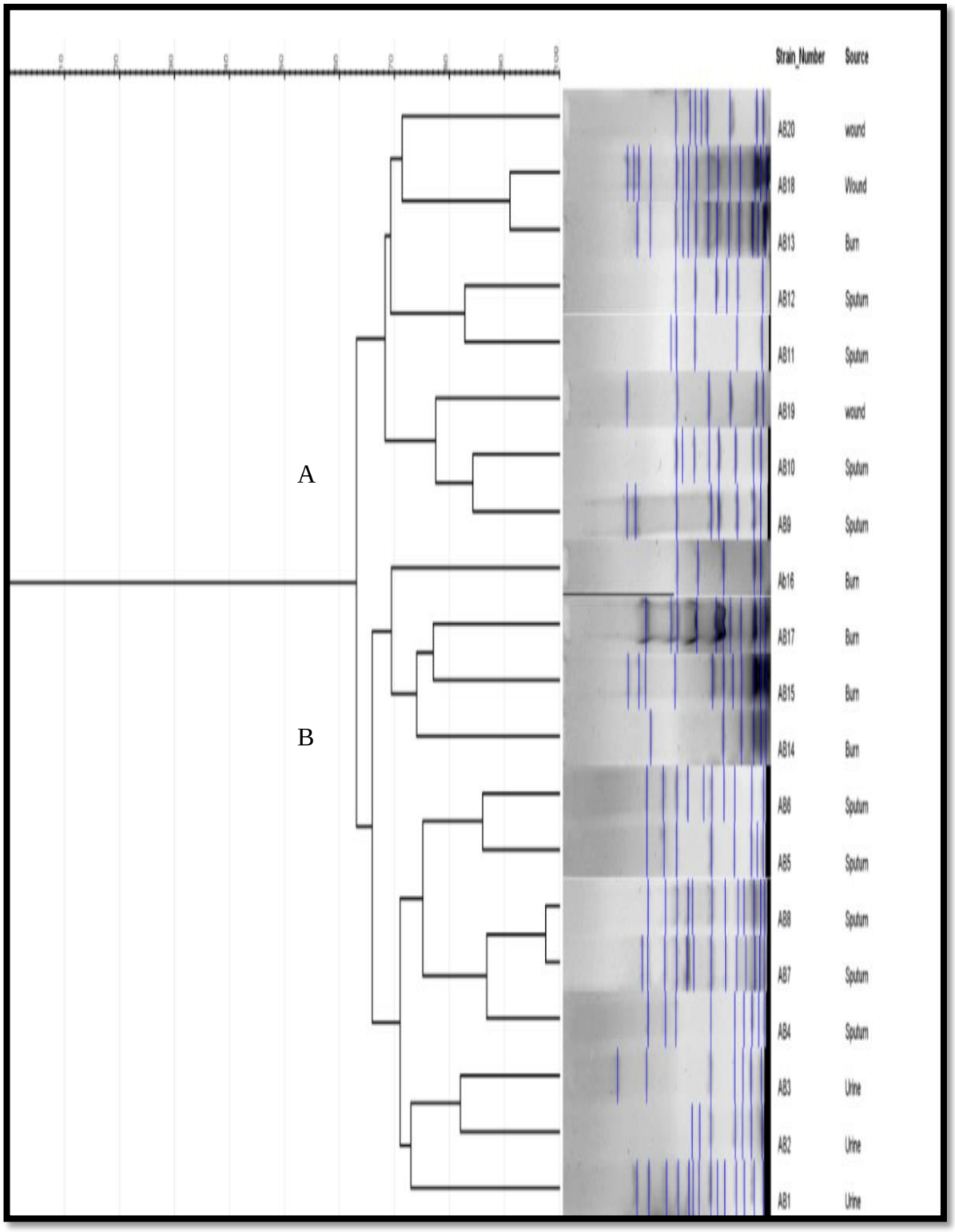

Fig 3-11: The ERIC-PCR-derived cladogram representing the relationship among 20 of *A. baumannii* in patients with different sample sources. Bar (above) represents the distance values. This cladogram was generated by Unweighted Pair Group Method with Arithmetic mean (UPGMA).

In this study, the ERIC Primer showed DNA polymorphism among *A. baumannii* isolates from different clinical sample, either in the occurrence of amplified fragment or in the variable genetic similarities of each isolate with the other.

The diversity could be due to the fact that they all were obtained from different sources, or due to the genetic instability of *A. baumannii.*

In the present study the genotyping of clinical *A. baumannii* isololates by ERic-PCR identified (20) distinct genetic profiles with different similarities value ranging from (20-80)% as shown in Fig. 3-12.

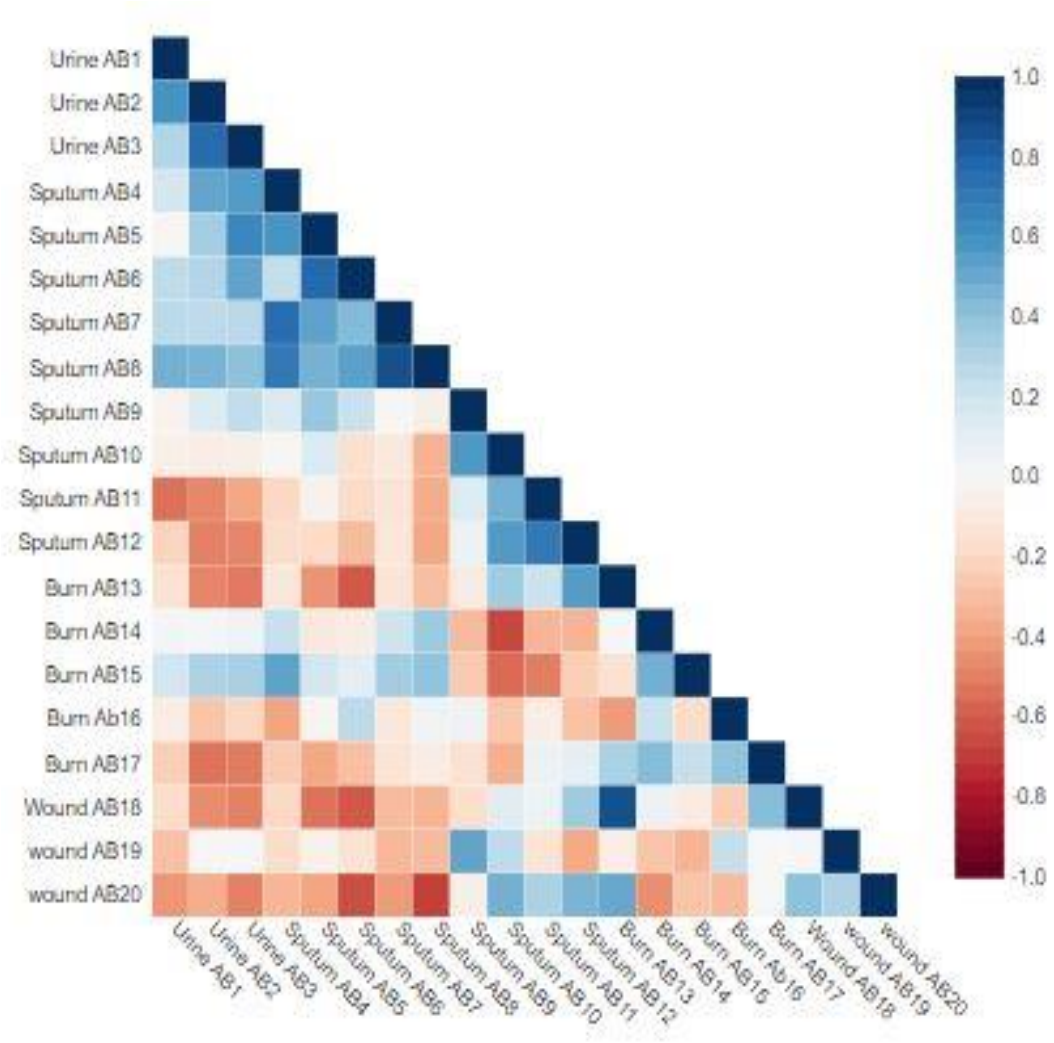

Fig. 3-12 : A Heat map represent the Genetic correlation similarity matrix among the studied 20 of *A. baumannii* strains using ERIC-PCR assay.

According to the results, this method indicated genetic diversity and heterogeneity among clinical isolates bacterium genome containing repeat sequence such as the ERIC sequence can be used as molecular biological tools to assess the Clonal variability of many bacteria isolates (Aljindan *et al.*, 2018)

ERIC - PCR finger printing is one of the fastest molecular typing techniques to differentiate between *A. baumannii* and other strain of Gram-negative bacteria responsible for hospital-acquired infection (Aljindan *et al.*, 2018)

ERIC- PER focused on the ability to discriminate between strains of the same or closely related species and also able to discriminate between members of different species. Also, the result of ERIC-PCR may play a role as a bacterial identification tool, providing more sensitive typing results than basic phenotypic typing methods (Otokunefor *et al.*, 2020)

By using ERIC-PCR fingerprinting genotype analysis (Aljindan *et al.*, 2018) found that 51 strains of *A. baumannii* were clustered into seven groups, while the remaining 8 were single strains. The genetic relatedness of *A. baumannii* isolated from admitted patients was high, indicating cross-transmission within the hospitalized patients.

Several studies have reported to identification of profiles in *A. baumannii* isolates as Falah *et al.* (2019) who identified 14 ERIC-PCR patterns among 80 isolates; and Heidari *et al.* (2018) who identified 20 ERIC-PCR types among 75 isolates.

ERIC-PCR are valuable typing methods for non- fermentative Gram - negative bacilli, and has pivotal role in understanding the essential mechanisms of *A. baumannii* infecting and discovering the relationship between bacterial species, also this method provides great potential to study bacterial Sequences because the sequences are longer and do not base on a specific region of the genome (Hazhirkamal *et al.*, 2021).

3.11 Detection of Box- element fingerprint in *A. baumannii*

By using Box primer, *A. baumannii* performed different DNA patterns with amplicons that gave a polymorphic band varied in size from (100-more than 2000) bp, the finger printing Patterns of the isolates were shown 15 bands on gel Electrophoresis as shown in figure 3-13.

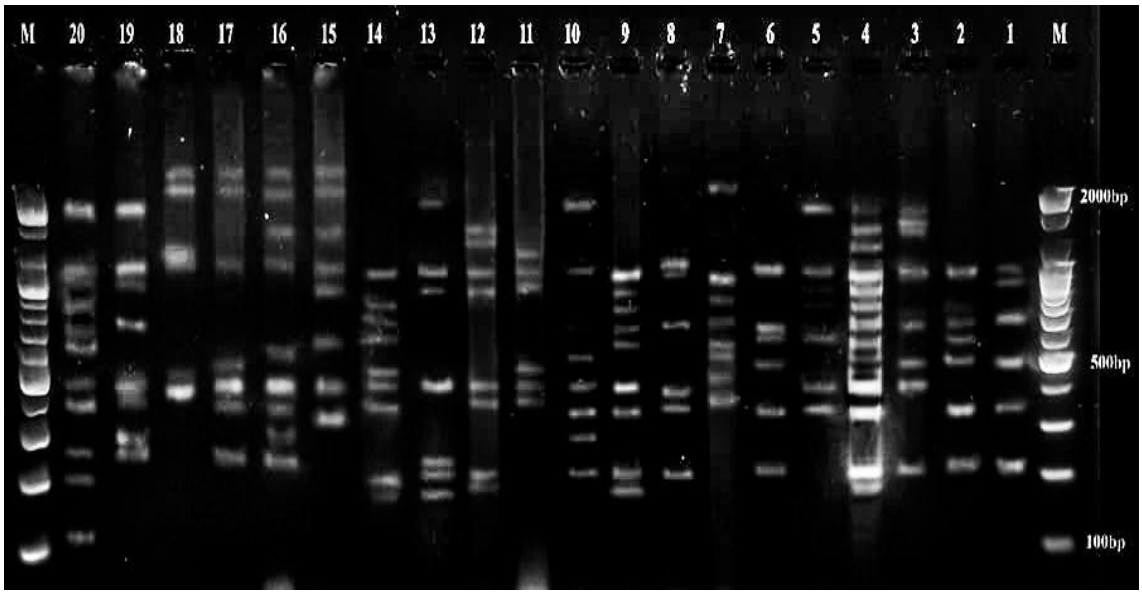

Fig. 3-13 :Agarose gel electrophoresis of PCR products BOX-PCR digital ,the electrophoresis was performed at 70 volt for 1 hr.

The data from Box Dendrogram of *A. baumannii* isolates were grouped into two main group, and among (20) collected strains of *A. baumannii*, the strains were grouped into 7 subgroups of which the group A contain one subgroup both of them belonging to sputum isolates and one unique belong to urine isolate, while group B contain 6 subgrouped as following: first one contains two sputum isolates, the second contain two wound and one burn, the third contain burn and sputum isolates whereas the fourth and fifth group both of them also contain one sample burn and one sputum and finally the sixth subgroup contain two urine isolates. on the other hand, there's five unique isolates including two sputum, two wound and one burn isolate as shown in figure 3-14 .

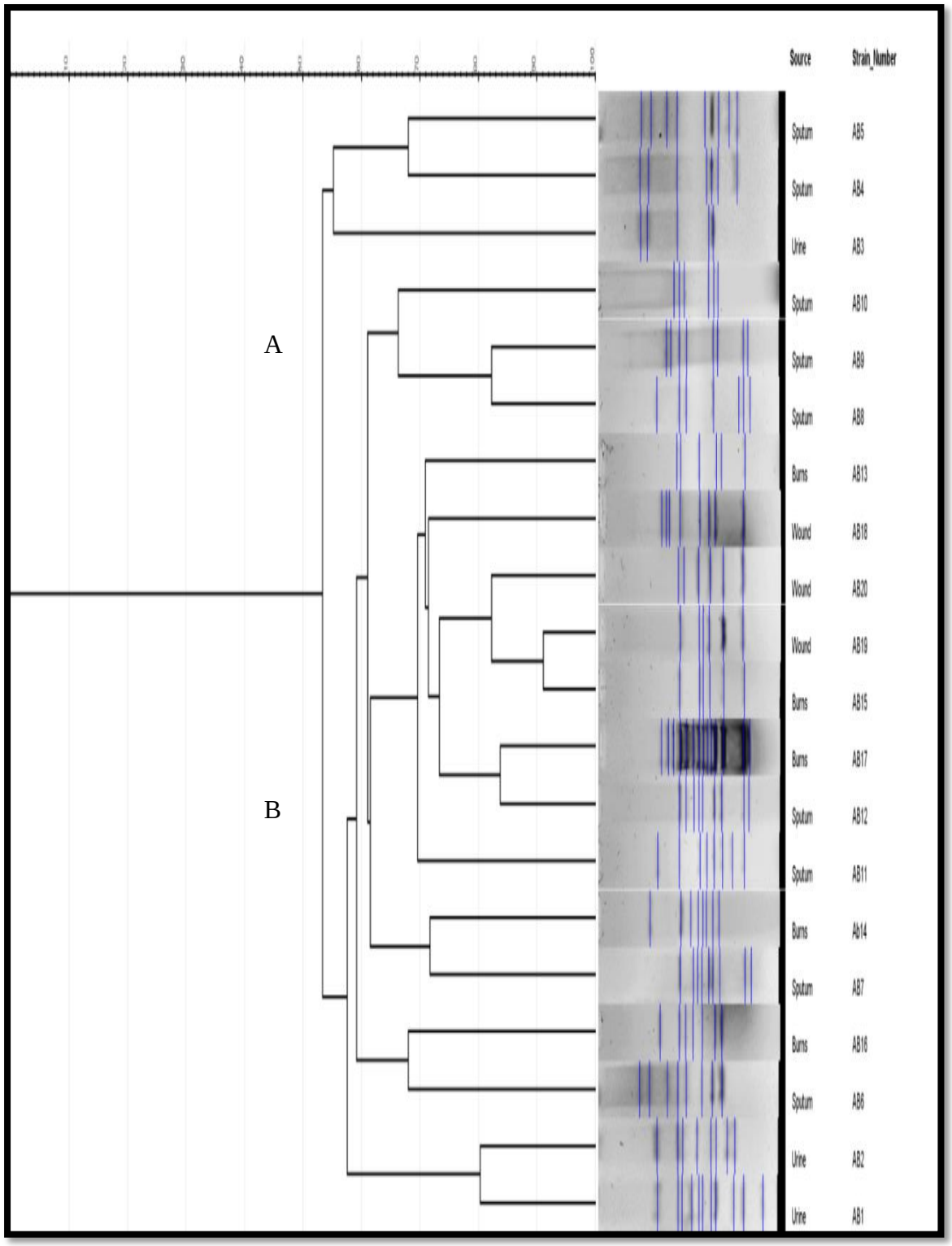

Fig. 3-14: The Box-PCR-derived cladogram representing the relationship among 20 of *A. baumannii* in patients with different sample sources. Bar (above) represents the distance values. This cladogram was generated by Unweighted Pair Group Method with Arithmetic mean (UPGMA).

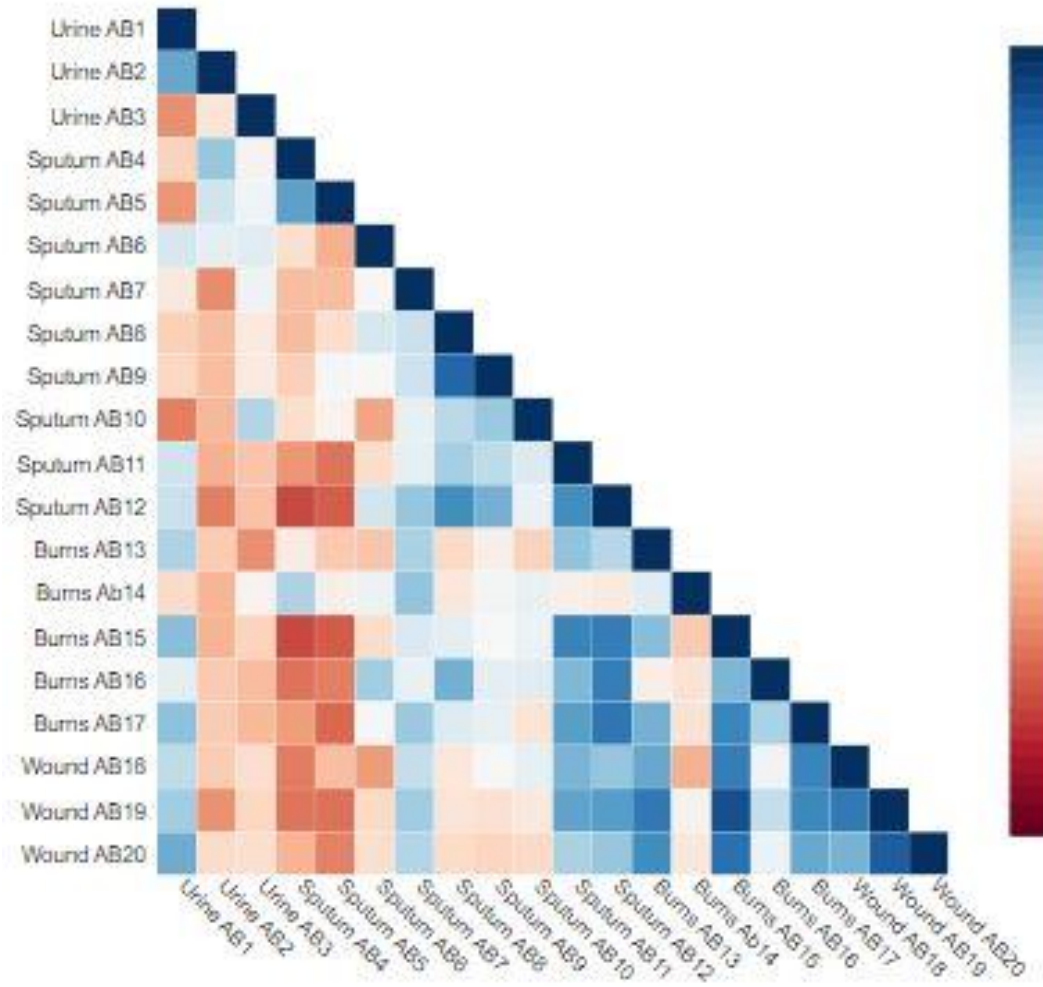

Fig. 3-15 : A Heat map represent the Genetic correlation similarity matrix among the studied 20 of *A. baumannii* strains using Box-PCR assay.

Most of the box sequences were encounter in close proximity to genes, suggesting their potential role as a regulatory element controlling coordinate virulence or competence - related gene expression; Box elements are the key element in adaptive bacterial evolution (Kheiri and Akhtari, 2017).

Study done by (Abdullah *et al.*, 2019) found that among the 20 *A. baumannii* isolates, the results showed the genetic relationship between *A. baumannii* clones, while 18 isolates contained different genotyping. It was

found that in spite of differences in the location and isolation sources of these isolates, a clear clonality was observed.

Genotyping method is a useful for detecting vector strains as well as identifying the epidemic between isolation and genetic relationships among isolates Alsaadi *et al.* (2021). The heterogeneity difference may demonstrate of environmental factor and the level of hospital hygiene on the distribution and genetic clonal formation variation.

Studies was demonstrated that Box - PCR method is a highly differentiated Power in study clinical isolation in the same genetic group indicating the transmission of pathogens from the hospital environment to patients as well as the distribution of pathogens in hospital environment (Abdullah *et al.*, 2019)

The different similarity in isolates in the same site of isolation due to they are gnomically different from each other which makes them distinguishable from one another.

The differences in similarity ratios between subspecies of the same species may be due to the different sources of sampling as well as possible mutations that result in genetic variation over time (Mohammed and Ahmad, 2021).

Conclusions

Conclusions

1- The highest percent of *A. baumannii* was found in sputum and followed by burns .

2- The isolate of *A. baumannii* were multidrug resistance to most of commonly used antibiotics.

3- It was found that there was a highly significant inhibition in growth of bacteria after exposure to the different dilution of nanoparticles $AgTIO_2$ and SWCN-OH.

4- It was observed that there was a highly significant inhibition in biofilm production of bacteria after exposure to the different concentration of $AgTIO_2$ and SWCN-OH nanoparticales.

5- It was found that *A. baumannii* isolates carry different types of CRISPR-cas associated gene according to the site of infection and the highest type was *cas* 3 gene(45%) follow by *cas* 6 gene(35%) .

6- The result in this study shows that the *A. baumannii* isolates were genotyping by ERIC-PCR and BOX PCR, these techniques are rapid and simple for routine screening and identification of clinical *A. baumannii* which are useful in epidemic potential.

Recommendations

Recommendations

1- Further studies the combination of nanoparticales and antibiotics against resistant hospital strains.

2- The utilization of new method in classification *A. baumannii* isolates like pulsed-field gel electrophoresis (PFGE) and Multilocus sequence typing (MLST) methods.

3- Further investigation in the field of study the genome of *A. baumannii* through fast evolving CRISPR/cas system and their role in controlling antibiotic resistance gene transfer and acquisition.

References

ABBY, S. S., NÉRON, B., MÉNAGER, H., TOUCHON, M. & ROCHA, E. P. 2014. MacSyFinder: a program to mine genomes for molecular systems with an application to CRISPR-Cas systems. *PloS one,* 9**,** e110726.

ABDI, S. N., GHOTASLOU, R., GANBAROV, K., MOBED, A., TANOMAND, A., YOUSEFI, M., ASGHARZADEH, M. & KAFIL, H. S. 2020. Acinetobacter baumannii efflux pumps and antibiotic resistance. *Infection and drug resistance,* 13**,** 423.

ABDULLAH, R. M., AHMED, R. Z. T. & AL-AZZAWI, S. N. A. 2019. Genotyping of Acinetobacter baumannii by using BOX Method. *Indian Journal of Forensic Medicine & Toxicology,* 13.

ABDUSSALAM-MOHAMMED, W. 2020. Comparison of chemical and biological properties of metal nanoparticles (Au, Ag), with metal oxide nanoparticles (ZnO-NPs) and their applications. *Advanced Journal of Chemistry-Section A,* 3**,** 192-210.

ABO-NEIMA, S. E., MOTAWEH, H. A. & ELSEHLY, E. M. 2020. Antimicrobial activity of functionalised carbon nanotubes against pathogenic microorganisms. *IET nanobiotechnology,* 14**,** 457-464.

AHMAD BARUDIN, N. H., SREEKANTAN, S., THONG, O. M. & SAHGAL, G. Antibacterial activity of Ag-TiO2 nanoparticles with various silver contents. Materials Science Forum, 2013. Trans Tech Publ, 238-245.

AHMED, S. 2017. *Molecular characterization of multidrug resistant Acinetobacter baumannii causing nosocomial infections.* [Sl: sn].

AHMED, S. S. & ALP, E. 2015. Genotyping methods for monitoring the epidemic evolution of A. baumannii strains. *The Journal of Infection in Developing Countries,* 9**,** 347-354.

AL-JUMAILI, A., ALANCHERRY, S., BAZAKA, K. & JACOB, M. V. J. M. 2017. Review on the antimicrobial properties of carbon nanostructures. 10**,** 1066.

References

AL-SHAMIRI, M. M., ZHANG, S., MI, P., LIU, Y., XUN, M., YANG, E., AI, L., HAN, L. & CHEN, Y. J. M. P. 2021. Phenotypic and genotypic characteristics of Acinetobacter baumannii enrolled in the relationship among antibiotic resistance, biofilm formation and motility. 155**,** 104922.

ALATROUNY, A. M., AMIN, M. A.-H. & SHABANA, H. S. 2013. Nosocomial infection with Acinetobacter spp. in intensive care unit patients. *Al-Azhar Assiut Medical Journal,* 10**,** 273- 283.

ALATROUNY, A. M., AMIN, M. A.-H. & SHABANA, H. S. 2020. prevalence of vancomycin resistant enterococci among patients with nosocomial infections in intensive care unit. *Al-Azhar Medical Journal,* 49**,** 1955-1964.

ALAVI, M., KARIMI, N. J. A. C., NANOMEDICINE, & BIOTECHNOLOGY 2018. Antiplanktonic, antibiofilm, antiswarming motility and antiquorum sensing activities of green synthesized Ag–TiO2, TiO2–Ag, Ag–Cu and Cu–Ag nanocomposites against multi-drug-resistant bacteria. 46**,** S399-S413.

ALIM, S., VEJAYAN, J., YUSOFF, M. M. & KAFI, A. 2018. Recent uses of carbon nanotubes & gold nanoparticles in electrochemistry with application in biosensing: A review. *Biosensors and Bioelectronics,* 121**,** 125-136.

ALJINDAN, R., ALSAMMAN, K. & ELHADI, N. 2018. ERIC-PCR genotyping of Acinetobacter baumannii isolated from different clinical specimens. *Saudi journal of medicine & medical sciences,* 6**,** 13.

ALMAGHRABI, M. K., JOSEPH, M. R., ASSIRY, M. M. & HAMID, M. E. 2018. Multidrug-resistant Acinetobacter baumannii: An emerging health threat in aseer region, Kingdom of Saudi Arabia. *Canadian Journal of Infectious Diseases and Medical Microbiology,* 2018.

References

ALMASAUDI, S. B. 2018. Acinetobacter spp. as nosocomial pathogens: Epidemiology and resistance features. *Saudi journal of biological sciences,* 25**,** 586-596.

ALSAADI, L. A. S., MOHSIN, I. H. & ALI, I. A. 2021. BOX-Genotyping of Carbapenem Resistant Acinetobacter baumannii Clinical Isolates from Diyala/Iraq. *Indian Journal of Forensic Medicine & Toxicology,* 15.

AMUDHAN, S., SEKAR, U., ARUNAGIRI, K. & SEKAR, B. 2011. OXA beta-lactamase-mediated carbapenem resistance in Acinetobacter baumannii. *Indian Journal of Medical Microbiology,* 29**,** 269-274.

ANTUNES, L., VISCA, P. & TOWNER, K. J. 2014. Acinetobacter baumannii: evolution of a global pathogen. *Pathogens and disease,* 71**,** 292-301.

AYENEW, Z., TIGABU, E., SYOUM, E., EBRAHIM, S., ASSEFA, D. & TSIGE, E. 2021. Multidrug resistance pattern of Acinetobacter species isolated from clinical specimens referred to the Ethiopian Public Health Institute: 2014 to 2018 trend anaylsis. *PloS one,* 16**,** e0250896.

BAILEY, W. & SCOTT, E. G. J. D. M. 1962. Diagnostic microbiology.

BARRANGOU, R., DUDLEY, E. G. J. A. R. O. F. S. & TECHNOLOGY 2016. CRISPR-based typing and next-generation tracking technologies. 7**,** 395-411.

BAYDA, S., ADEEL, M., TUCCINARDI, T., CORDANI, M. & RIZZOLIO, F. 2020. The history of nanoscience and nanotechnology: From chemical–physical applications to nanomedicine. *Molecules,* 25**,** 112.

BEABOUT, K., HAMMERSTROM, T. G., PEREZ, A. M., MAGALHÃES, B. F., PRATER, A. G., CLEMENTS, T. P., ARIAS, C. A., SAXER, G. & SHAMOO, Y. 2015. The ribosomal S10

protein is a general target for decreased tigecycline susceptibility. *Antimicrobial agents and chemotherapy,* 59**,** 5561-5566.

BELLO, A. & DINGLE, T. C. J. C. M. N. 2018. What's that resistance mechanism? Understanding genetic determinants of gram-negative bacterial resistance. 40**,** 165-174.

BERGOGNE-BÉRÉZIN, E., JOLY-GUILLOU, M.-L. & TOWNER, K. 2020. Introduction—History and Importance of Acinetobacter spp., Role in Infections, Treatment and Cost Implications. *Acinetobacter*. CRC Press.

BILUNG, L. M., PUI, C. F., SU'UT, L. & APUN, K. 2018. Evaluation of BOX-PCR and ERIC-PCR as molecular typing tools for pathogenic Leptospira. *Disease markers,* 2018.

BISWAS, I. & RATHER, P. N. 2019. *Acinetobacter baumannii*, Springer.

BOJKOVIC, J., RICHIE, D. L., SIX, D. A., RATH, C. M., SAWYER, W. S., HU, Q. & DEAN, C. R. 2015. Characterization of an Acinetobacter baumannii lptD deletion strain: permeability defects and response to inhibition of lipopolysaccharide and fatty acid biosynthesis. *Journal of bacteriology,* 198**,** 731-741.

BROOKS, G., CARROLL, K., BUTEL, J., MORSE, S. & MIETZNER, T. 2013. Jawetz, Melnick and Adelberg, s medical microbiology, 26th edn.(The McGraw-Hill Companies: USA).

BROSSARD, K. A. & CAMPAGNARI, A. A. 2012. The Acinetobacter baumannii biofilm-associated protein plays a role in adherence to human epithelial cells. *Infection and immunity,* 80**,** 228-233.

BROWN, A. & SMITH, H. 2014. *Benson's Microbiological Applications, Laboratory Manual in General Microbiology, Short Version*, McGraw-Hill Education.

BUTIUC-KEUL, A., FARKAS, A., CARPA, R. & IORDACHE, D. 2022. CRISPR-Cas system: the powerful modulator of accessory genomes in prokaryotes. *Microbial Physiology,* 32**,** 2-17.

References

BUTLER, D. A., BIAGI, M., TAN, X., QASMIEH, S., BULMAN, Z. P. & WENZLER, E. 2019. Multidrug resistant Acinetobacter baumannii: resistance by any other name would still be hard to treat. *Current infectious disease reports*, 21**,** 1-17.

CHINE, W., MELLIT, A., LUGHI, V., MALEK, A., SULLIGOI, G. & PAVAN, A. M. J. R. E. 2016. A novel fault diagnosis technique for photovoltaic systems based on artificial neural networks. 90**,** 501-512.

CHLUMSKY, O., PURKRTOVA, S., MICHOVA, H., SVARCOVA, V., SLEPICKA, P., FAJSTAVR, D., ULBRICH, P. & DEMNEROVA, K. 2020. The effect of gold and silver nanoparticles, chitosan and their combinations on bacterial biofilms of food-borne pathogens. *Biofouling*, 36**,** 222-233.

CINAR, H. N., GOPINATH, G., MURPHY, H. R., ALMERIA, S., DURIGAN, M., CHOI, D., JANG, A., KIM, E., KIM, R. & CHOI, S. 2020. Molecular typing of Cyclospora cayetanensis in produce and clinical samples using targeted enrichment of complete mitochondrial genomes and next-generation sequencing. *Parasites & vectors,* 13**,** 1-12.

CLARK, N. M., ZHANEL, G. G. & LYNCH III, J. P. 2016. Emergence of antimicrobial resistance among Acinetobacter species: a global threat. *Current opinion in critical care*, 22**,** 491-499.

CLINICAL & M100, L. S. I. J. T. E. C. S. 2020. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing.

COLQUHOUN, J. M. & RATHER, P. N. 2020. Insights into mechanisms of biofilm formation in Acinetobacter baumannii and implications for uropathogenesis. *Frontiers in cellular and infection microbiology*, 10**,** 253.

References

COLQUHOUN, J. M., RATHER, P. N. J. F. I. C. & MICROBIOLOGY, I. 2020. Insights into mechanisms of biofilm formation in Acinetobacter baumannii and implications for uropathogenesis. 10**,** 253.

CONLY, J. & JOHNSTON, B. 2005. Where are all the new antibiotics? The new antibiotic paradox. *Canadian Journal of Infectious Diseases and Medical Microbiology,* 16**,** 159-160.

DA PAZ PEREIRA, J. N., DE ANDRADE, C. A. D. N., DA COSTA LIMA, J. L., DE LIMA NETO, R. G., DE ARAÚJO, P. S. R. & MACIEL, M. A. V. J. C. M. 2020. Clonal dissemination of clinical isolates of acinetobacter baumannii carriers of 16S rRNA methylase genes in an oncological hospital in Recife, Brazil. 77**,** 32-39.

DA SILVA, B. L., ABUÇAFY, M. P., MANAIA, E. B., JUNIOR, J. A. O., CHIARI-ANDRÉO, B. G., PIETRO, R. C. R. & CHIAVACCI, L. A. 2019. Relationship between structure and antimicrobial activity of zinc oxide nanoparticles: An overview. *International journal of nanomedicine,* 14**,** 9395.

DALLO, S. F. & WEITAO, T. 2010. Insights into acinetobacter war-wound infections, biofilms, and control. *Advances in skin & wound care,* 23**,** 169-174.

DESCHAGHT, P., VAN SIMAEY, L., DECAT, E., VAN MECHELEN, E., BRISSE, S. & VANEECHOUTTE, M. 2011. Rapid genotyping of Achromobacter xylosoxidans, Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa and Stenotrophomonas maltophilia isolates using melting curve analysis of RAPD-generated DNA fragments (McRAPD). *Research in microbiology,* 162**,** 386-392.

DEWACHI, O. 2019. Iraqibacter and the Pathologies of Intervention. *Middle East Report,* 290**,** 8-12.

References

DI VENANZIO, G., FLORES-MIRELES, A. L., CALIX, J. J., HAURAT, M. F., SCOTT, N. E., PALMER, L. D., POTTER, R. F., HIBBING, M. E., FRIEDMAN, L. & WANG, B. 2019. Urinary tract colonization is enhanced by a plasmid that regulates uropathogenic Acinetobacter baumannii chromosomal genes. *Nature communications,* 10**,** 1-13.

DIANCOURT, L., PASSET, V., NEMEC, A., DIJKSHOORN, L. & BRISSE, S. 2010. The population structure of Acinetobacter baumannii: expanding multiresistant clones from an ancestral susceptible genetic pool. *PloS one,* 5**,** e10034.

DÍEZ-PASCUAL, A. M. 2020. Recent progress in antimicrobial nanomaterials. *Nanomaterials,* 10**,** 2315.

DIMPLE, R., NUPUR, S., MAHAWAL, B., ANKIT, K. & AJAY, P. 2016. Speciation and antibiotic resistance pattern of Acinetobacter species in a tertiary care hospital in Uttarakhand. *International Journal of Medical Research & Health Sciences,* 5**,** 89-96.

DOI, Y., MURRAY, G. L. & PELEG, A. Y. Acinetobacter baumannii: evolution of antimicrobial resistance—treatment options. Seminars in respiratory and critical care medicine, 2015. Thieme Medical Publishers, 085-098.

DONG, L., HENDERSON, A. & FIELD, C. J. J. O. N. 2012. Antimicrobial activity of single-walled carbon nanotubes suspended in different surfactants. 2012.

DOS SANTOS, B. A., DE OLIVEIRA, J. D. S., PARMANHANI-DA-SILVA, B. M., RIBEIRO, R. L., TEIXEIRA, L. M., NEVES, F. P. G. J. I., GENETICS & EVOLUTION 2020. CRISPR elements and their association with antimicrobial resistance and virulence genes among vancomycin-resistant and vancomycin-susceptible enterococci recovered from human and food sources. 80**,** 104183.

References

ESPINAL, P., MARTI, S. & VILA, J. 2012. Effect of biofilm formation on the survival of Acinetobacter baumannii on dry surfaces. *Journal of Hospital Infection*, 80**,** 56-60.

EZE, E. C., CHENIA, H. Y. & EL ZOWALATY, M. E. 2018. Acinetobacter baumannii biofilms: effects of physicochemical factors, virulence, antibiotic resistance determinants, gene regulation, and future antimicrobial treatments. *Infection and drug resistance*, 11**,** 2277.

FALAH, F., SHOKOOHIZADEH, L. & ADABI, M. 2019. Molecular identification and genotyping of Acinetobacter baumannii isolated from burn patients by PCR and ERIC-PCR. *Scars, burns & healing*, 5**,** 2059513119831369.

FAM, S. Y., CHEE, C. F., YONG, C. Y., HO, K. L., MARIATULQABTIAH, A. R. & TAN, W. S. 2020. Stealth coating of nanoparticles in drug-delivery systems. *Nanomaterials*, 10**,** 787.

FERNÁNDEZ, L. & HANCOCK, R. E. 2012. Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. *Clinical microbiology reviews*, 25**,** 661-681.

FORBES, B. A., SAHM, D. F. & WEISSFELD, A. S. 2007. *Study guide for Bailey & Scott's diagnostic microbiology*, Mosby Maryland Heights, MO, USA.

GARRETT, S. C. 2021. Pruning and tending immune memories: spacer dynamics in the CRISPR array. *Frontiers in Microbiology*, 12**,** 739.

GEDEFIE, A., DEMSIS, W., ASHAGRIE, M., KASSA, Y., TESFAYE, M., TILAHUN, M., BISETEGN, H. & SAHLE, Z. 2021. Acinetobacter baumannii biofilm formation and its role in disease pathogenesis: A review. *Infection and Drug Resistance,* 14**,** 3711.

GODOY-GALLARDO, M., ECKHARD, U., DELGADO, L. M., DE ROO PUENTE, Y. J., HOYOS-NOGUÉS, M., GIL, F. J. & PEREZ, R. A. 2021. Antibacterial approaches in tissue engineering using metal ions

and nanoparticles: From mechanisms to applications. *Bioactive Materials,* 6**,** 4470-4490.

GOLD, K., SLAY, B., KNACKSTEDT, M. & GAHARWAR, A. K. 2018. Antimicrobial activity of metal and metal-oxide based nanoparticles. *Advanced Therapeutics,* 1**,** 1700033.

GRAVES JR, J. L., TAJKARIMI, M., CUNNINGHAM, Q., CAMPBELL, A., NONGA, H., HARRISON, S. H. & BARRICK, J. E. 2015. Rapid evolution of silver nanoparticle resistance in Escherichia coli. *Frontiers in genetics,* 6**,** 42.

GULATI, K., SCIMECA, J.-C., IVANOVSKI, S. & VERRON, E. 2021. Double-edged sword: therapeutic efficacy versus toxicity evaluations of doped titanium implants. *Drug Discovery Today,* 26**,** 2734-2742.

GUO, T., SUN, X., LI, M., WANG, Y., JIAO, H. & LI, G. J. F. I. M. 2022. The Involvement of the csy1 Gene in the Antimicrobial Resistance of Acinetobacter baumannii. 9.

HALL-STOODLEY, L. & STOODLEY, P. 2005. Biofilm formation and dispersal and the transmission of human pathogens. *Trends in microbiology,* 13**,** 7-10.

HAMIDIAN, M. & NIGRO, S. J. J. M. G. 2019. Emergence, molecular mechanisms and global spread of carbapenem-resistant Acinetobacter baumannii. 5.

HAMZA, M. & YAAQOOB, L. J. T. I. J. O. A. S. 2020. EVALUATION THE EFFECT OF GREEN SYNTHESIS TITANIUM DIOXIDE NANOPARTICLES ON ACINETOBACTER BAUMANNII ISOLATES. 51**,** 1486-1495.

HARLEY, J. P. & PRESCOTT, L. M. 1996. Laboratory exercises in microbiology.

HAUCK, Y., SOLER, C., JAULT, P., MÉRENS, A., GÉROME, P., NAB, C. M., TRUEBA, F., BARGUES, L., THIEN, H. V. & VERGNAUD, G. 2012. Diversity of Acinetobacter baumannii in

four French military hospitals, as assessed by multiple locus variable number of tandem repeats analysis.

HAZHIRKAMAL, M., ZAREI, O., MOVAHEDI, M., KARAMI, P., SHOKOOHIZADEH, L. & TAHERI, M. 2021. Molecular typing, biofilm production, and detection of carbapenemase genes in multidrug-resistant Acinetobacter baumannii isolated from different infection sites using ERIC-PCR in Hamadan, west of Iran. *BMC Pharmacology and Toxicology,* 22**,** 1-7.

HEIDARI, H., HALAJI, M., TAJI, A., KAZEMIAN, H., ABADI, M. S. S., SISAKHT, M. T. & EBRAHIM-SARAIE, H. S. 2018. Molecular analysis of drug-resistant Acinetobacter baumannii isolates by ERIC-PCR. *Meta Gene,* 17**,** 132-135.

HØIBY, N., BJARNSHOLT, T., GIVSKOV, M., MOLIN, S. & CIOFU, O. 2010. Antibiotic resistance of bacterial biofilms. *International journal of antimicrobial agents,* 35**,** 322-332.

HOOD, M. I., JACOBS, A. C., SAYOOD, K., DUNMAN, P. M. & SKAAR, E. P. 2010. Acinetobacter baumannii increases tolerance to antibiotics in response to monovalent cations. *Antimicrobial agents and chemotherapy,* 54**,** 1029-1041.

HORVATH, P., ROMERO, D. A., COÛTÉ-MONVOISIN, A.-C., RICHARDS, M., DEVEAU, H., MOINEAU, S., BOYAVAL, P., FREMAUX, C. & BARRANGOU, R. 2008. Diversity, activity, and evolution of CRISPR loci in Streptococcus thermophilus. *Journal of bacteriology,* 190**,** 1401-1412.

IBRAHIM, M. E., ALGAK, T. B., ABBAS, M., ELAMIN, B. K. J. E. & MEDICINE, T. 2021. Emergence of bla TEM, bla CTX-M, bla SHV and bla OXA genes in multidrug-resistant Enterobacteriaceae and Acinetobacter baumannii in Saudi Arabia. 22**,** 1-11.

References

IRAVANI, S., KORBEKANDI, H., MIRMOHAMMADI, S. V. & MEKANIK, H. 2014. Plants in nanoparticle synthesis. *Reviews in Advanced Sciences and Engineering,* 3**,** 261-274.

IYER, R., MOUSSA, S. H., DURAND-REVILLE, T. F., TOMMASI, R. & MILLER, A. 2017. Acinetobacter baumannii OmpA is a selective antibiotic permeant porin. *ACS infectious diseases,* 4**,** 373-381.

JAFARI, A. J., ROSTAMI, R. & GHAINY, G. 2018. Advance in bioaerosol removal technologies; a review. *Iranian Journal of Health, Safety and Environment,* 5**,** 1007-1016.

JARURATANASIRIKUL, S., NITCHOT, W., WONGPOOWARAK, W., SAMAENG, M. & NAWAKITRANGSAN, M. 2019. Population pharmacokinetics and Monte Carlo simulations of sulbactam to optimize dosage regimens in patients with ventilator-associated pneumonia caused by Acinetobacter baumannii. *European Journal of Pharmaceutical Sciences,* 136**,** 104940.

JASSIM, K. A., GHAIMA, K. K., SAADEDIN, S. M. K. J. A. J. O. C. M. & INFECTION 2016. AdeABC efflux pump genes in multidrug resistant Acinetobacter baumannii isolates. 3**,** 40898-40898.

JHA, C., GHOSH, S., GAUTAM, V., MALHOTRA, P. & RAY, P. 2017. In vitro study of virulence potential of Acinetobacter baumannii outer membrane vesicles. *Microbial pathogenesis,* 111**,** 218-224.

JONES, D. L., LEROY, P., UNOSON, C., FANGE, D., ĆURIĆ, V., LAWSON, M. J. & ELF, J. 2017. Kinetics of dCas9 target search in Escherichia coli. *Science,* 357**,** 1420-1424.

KAMOSHIDA, G., AKAJI, T., TAKEMOTO, N., SUZUKI, Y., SATO, Y., KAI, D., HIBINO, T., YAMAGUCHI, D., KIKUCHI-UEDA, T. & NISHIDA, S. J. F. I. M. 2020. Lipopolysaccharide-deficient acinetobacter baumannii due to colistin resistance is killed by neutrophil-produced lysozyme. 11**,** 573.

References

KANJ, S. S. & KANAFANI, Z. A. Current concepts in antimicrobial therapy against resistant gram-negative organisms: extended-spectrum β-lactamase–producing enterobacteriaceae, carbapenem-resistant enterobacteriaceae, and multidrug-resistant Pseudomonas aeruginosa. Mayo Clinic Proceedings, 2011. Elsevier, 250-259.

KARAH, N., SAMUELSEN, Ø., ZARRILLI, R., SAHL, J. W., WAI, S. N. & UHLIN, B. E. 2015. CRISPR-cas subtype I-Fb in Acinetobacter baumannii: evolution and utilization for strain subtyping. *PLoS One*, 10**,** e0118205.

KAUR, A., SINGH, S., GILL, A. K., KAUR, N. & MAHAJAN, A. J. B. 2016. Isolation of Acinetobacter baumannii and it's antimicrobial resistance pattern in an intensive care unit (ICU) of a tertiary care hospital. 146**,** 4.

KHALID, P., HUSSAIN, M., SUMAN, V. & ARUN, A. J. I. J. O. A. E. R. 2016. Toxicology of carbon nanotubes-a review. 11**,** 148-157.

KHARISOV, B. I., PÉREZ, B. O., DE CASAS ORTIZ, E. G., KHARISSOVA, O. V., CORONA, M. D. R. C., PÉREZ, V. M. J. & DIAS, R. 2013. Synthesis of Iron-containing Nanomaterials by "Greener" Methods and Their Use for Disinfection of Water. *MRS Online Proceedings Library (OPL)*, 1558.

KHEIRI, R. & AKHTARI, L. J. K. U. V. F. D. 2017. Clonal heterogeneity and efficacy of BOX and (GTG) 5 fingerprinting methods for molecular typing of Escherichia coli isolated from chickens in IRI. 23**,** 219-225.

KONG, B. H., HANIFAH, Y. A., YUSOF, M. Y. M. & THONG, K. L. J. J. J. O. I. D. 2011. Antimicrobial susceptibility profiling and genomic diversity of multidrug-resistant Acinetobacter baumannii isolates from a teaching hospital in Malaysia. 64**,** 337-340.

References

KOONIN, E. V. & MAKAROVA, K. S. 2019. Origins and evolution of CRISPR-Cas systems. *Philosophical Transactions of the Royal Society B,* 374**,** 20180087.

KOTŁOWSKI, R., NOWAK-ZALESKA, A. & WĘGRZYN, G. 2021. Discrimination of hospital isolates of Acinetobacter baumannii using repeated sequences and whole genome alignment differential analysis. *Journal of Applied Genetics,* 62**,** 511-521.

LAHIRI, K., MANI, N. & PURAI, S. 2004. Acinetobacter spp as nosocomial pathogen: Clinical significance and antimicrobial sensitivity. *Medical Journal Armed Forces India,* 60**,** 7-10.

LEE, J.-Y., JEONG, M.-C., JEON, D., LEE, Y., LEE, W. C., KIM, Y. J. B. & CHEMISTRY, M. 2017. Structure-activity relationship-based screening of antibiotics against Gram-negative Acinetobacter baumannii. 25**,** 372-380.

LEE, P. Y., COSTUMBRADO, J., HSU, C.-Y. & KIM, Y. H. J. J. 2012. Agarose gel electrophoresis for the separation of DNA fragments. e3923.

LEUNGTONGKAM, U., THUMMEEPAK, R., KITTI, T., TASANAPAK, K., WONGWIGKARN, J., STYLES, K. M., WELLINGTON, E. M., MILLARD, A. D., SAGONA, A. P. & SITTHISAK, S. 2020. Genomic analysis reveals high virulence and antibiotic resistance amongst phage susceptible Acinetobacter baumannii. *Scientific reports,* 10**,** 1-11.

LI, A., WANG, L., LI, M., XU, Z., LI, P., ZHANG, Z. J. D. J. O. N. & BIOSTRUCTURES 2014. The antibacterial activity of covalent-functionalized carbon nanotubes against Escherichia coli. 9**,** 599-607.

LI, R., YU, H. & CHEN, X. 2020. Recent progress in chemical synthesis of bacterial surface glycans. *Current opinion in chemical biology,* 58**,** 121-136.

References

LI, Z., DING, Z., LIU, Y., JIN, X., XIE, J., LI, T., ZENG, Z., WANG, Z., LIU, J. J. I. & RESISTANCE, D. 2021. Phenotypic and genotypic characteristics of biofilm formation in clinical isolates of Acinetobacter baumannii. 14**,** 2613.

LI, Z.-T., ZHANG, R.-L., BI, X.-G., XU, L., FAN, M., XIE, D., XIAN, Y., WANG, Y., LI, X.-J. & WU, Z.-D. 2015. Outer membrane vesicles isolated from two clinical Acinetobacter baumannii strains exhibit different toxicity and proteome characteristics. *Microbial pathogenesis,* 81**,** 46-52.

LIN, T.-L., PAN, Y.-J., HSIEH, P.-F., HSU, C.-R., WU, M.-C. & WANG, J.-T. 2016. Imipenem represses CRISPR-Cas interference of DNA acquisition through H-NS stimulation in Klebsiella pneumoniae. *Scientific reports,* 6**,** 1-10.

LIU, Z., DONG, H., CUI, Y., CONG, L. & ZHANG, D. 2020. Application of different types of CRISPR/Cas-based systems in bacteria. *Microbial cell factories,* 19**,** 1-14.

LÓPEZ DE DICASTILLO, C., PATIÑO, C., GALOTTO, M. J., PALMA, J. L., ALBURQUENQUE, D. & ESCRIG, J. 2018. Novel antimicrobial titanium dioxide nanotubes obtained through a combination of atomic layer deposition and electrospinning technologies. *Nanomaterials,* 8**,** 128.

LOUWEN, R., STAALS, R. H., ENDTZ, H. P., VAN BAARLEN, P. & VAN DER OOST, J. 2014. The role of CRISPR-Cas systems in virulence of pathogenic bacteria. *Microbiology and Molecular Biology Reviews,* 78**,** 74-88.

LUKOVIC, B., GAJIC, I., DIMKIC, I., KEKIC, D., ZORNIC, S., POZDER, T., RADISAVLJEVIC, S., OPAVSKI, N., KOJIC, M., RANIN, L. J. A. R. & CONTROL, I. 2020. The first nationwide multicenter study of Acinetobacter baumannii recovered in Serbia:

emergence of OXA-72, OXA-23 and NDM-1-producing isolates. 9**,** 1-12.

LUPO, A., HAENNI, M. & MADEC, J.-Y. 2018. Antimicrobial resistance in Acinetobacter spp. and Pseudomonas spp. *Microbiology spectrum,* 6**,** 6.3. 01.

MAHICH, S., ANGURANA, S. K., SUTHAR, R., SUNDARAM, V., MUNDA, V. S. & GAUTAM, V. J. T. I. J. O. P. 2021. Acinetobacter sepsis among out-born neonates admitted to neonatal unit in pediatric emergency of a tertiary care hospital in North India. 88**,** 127-133.

MAKAROVA, K. S. & KOONIN, E. V. 2015. Annotation and classification of CRISPR-Cas systems. *CRISPR***,** 47-75.

MAKAROVA, K. S., WOLF, Y. I. & KOONIN, E. V. 2013. The basic building blocks and evolution of CRISPR–Cas systems. *Biochemical Society Transactions,* 41**,** 1392-1400.

MAKAROVA, K. S., WOLF, Y. I. & KOONIN, E. V. J. T. C. J. 2018. Classification and nomenclature of CRISPR-Cas systems: where from here? 1**,** 325-336.

MALEK, I., SCHABER, C., HEINLEIN, T., SCHNEIDER, J., GORB, S. & SCHMITZ, R. J. J. O. M. C. B. 2016. Vertically aligned multi walled carbon nanotubes prevent biofilm formation of medically relevant bacteria. 4**,** 5228-5235.

MALEKI, A., VANDYOUSEFI, J., MIRZAIE, Z., GHAFOURIAN, S., KAZEMIAN, H. & SADEGHIFARD, N. 2018. Molecular analysis of the isolates of Acinetobacter baumannii isolated from Tehran hospitals using ERIC-PCR method. *Modern Medical Laboratory Journal,* 1**,** 12-16.

MANGAS, E. L., RUBIO, A., ÁLVAREZ-MARÍN, R., LABRADOR-HERRERA, G., PACHÓN, J., PACHÓN-IBÁÑEZ, M. E., DIVINA, F. & PÉREZ-PULIDO, A. J. 2019. Pangenome of Acinetobacter

baumannii uncovers two groups of genomes, one of them with genes involved in CRISPR/Cas defence systems associated with the absence of plasmids and exclusive genes for biofilm formation. *Microbial genomics,* 5.

MARCINKIEWICZ, J., STRUS, M. & PASICH, E. 2013. Antibiotic resistance: a" dark side" of biofilm-associated chronic infections. *Polskie Archiwum Medycyny Wewnętrznej= Polish Archives of Internal Medicine,* 123.

MARTÍN-ASPAS, A., GUERRERO-SÁNCHEZ, F. M., GARCÍA-COLCHERO, F., RODRÍGUEZ-ROCA, S. & GIRÓN-GONZÁLEZ, J.-A. 2018. Differential characteristics of Acinetobacter baumannii colonization and infection: risk factors, clinical picture, and mortality. *Infection and drug resistance,* 11**,** 861.

MASTUNAGA, T. 1985. Photoelectrochemical sterilization of microbial cells by semiconductor powders. *FEMS Microbiol. Lett.,* 29**,** 211-214.

MCDONALD, J. J. B., MD: AVAILABLE HTTP://WWW. BIOSTATHANDBOOK. COM/TRANSFORMATION. HTML 2014. Handbook of Biological Statistics 3rd ed Sparky House Publishing.

MCGANN, P., COURVALIN, P., SNESRUD, E., CLIFFORD, R. J., YOON, E.-J., ONMUS-LEONE, F., ONG, A. C., KWAK, Y. I., GRILLOT-COURVALIN, C. & LESHO, E. 2014. Amplification of aminoglycoside resistance gene aphA1 in Acinetobacter baumannii results in tobramycin therapy failure. *MBio,* 5**,** e00915-14.

MESCHIARI, M., KALECI, S., ORLANDO, G., SELMI, S., SANTORO, A., BACCA, E., MENOZZI, M., FRANCESCHINI, E., PUZZOLANTE, C. & BEDINI, A. 2021. Risk factors for nosocomial rectal colonization with carbapenem-resistant

Acinetobacter baumannii in hospital: a matched case–control study. *Antimicrobial Resistance & Infection Control,* 10**,** 1-11.

MEUMANN, E. M., ANSTEY, N. M., CURRIE, B. J., PIERA, K. A., KENYON, J. J., HALL, R. M., DAVIS, J. S. & SAROVICH, D. S. 2019. Genomic epidemiology of severe community-onset Acinetobacter baumannii infection. *Microbial genomics,* 5.

MOHAMMED, G. & AHMAD, N. H. 2021. Detection of some virulance genes and variation of Acinetobacter Baumannii. *Middle East Journal of Science,* 7**,** 64-79.

MOHAMMED, S. H., AHMED, M. M., ABD ALAMEER ABD ALREDAA, N., HAIDER ABD ALABBAS, H., MOHAMMAD ALI, Z. D., ABED AL-WAHAB, Z. Z., ALI MOHSIN, Z., JALIL JASIM MOHAMMED, Z., ABD AL HAMZA MOHAMMED, Z. & YAHYA ABID ZAID, N. J. I. J. O. M. M. 2022. Prevalence of Acinetobacter Species Isolated from Clinical Samples Referred to Al-Kafeel Hospital, Iraq and Their Antibiotic Susceptibility Patterns from 2017-2021. 16**,** 76-82.

MOHID, S. A., GHORAI, A., ILYAS, H., MROUE, K. H., NARAYANAN, G., SARKAR, A., RAY, S. K., BISWAS, K., BERA, A. K. & MALMSTEN, M. 2019. Application of tungsten disulfide quantum dot-conjugated antimicrobial peptides in bio-imaging and antimicrobial therapy. *Colloids and Surfaces B: Biointerfaces,* 176**,** 360-370.

MONEM, S., FURMANEK-BLASZK, B., ŁUPKOWSKA, A., KUCZYŃSKA-WIŚNIK, D., STOJOWSKA-SWĘDRZYŃSKA, K. & LASKOWSKA, E. 2020. Mechanisms protecting Acinetobacter baumannii against multiple stresses triggered by the host immune response, antibiotics and outside-host environment. *International Journal of Molecular Sciences,* 21**,** 5498.

References

MORADPOOR, H., SAFAEI, M., GOLSHAH, A., MOZAFFARI, H. R., SHARIFI, R., IMANI, M. M. & MOBARAKEH, M. S. 2021. Green synthesis and antifungal effect of titanium dioxide nanoparticles on oral Candida albicans pathogen. *Inorganic Chemistry Communications,* 130**,** 108748.

MUBDIR, D. M., AL-SHUKRI, M. S. & GHALEB, R. A. 2021. Antimicrobial Activity of Gold Nanoparticles and SWCNT-COOH on Viability of Pseudomonas aeruginosa. *Annals of the Romanian Society for Cell Biology***,** 5507-5513.

MUFLIKHUN, M. A., FROMMELT, M. C., FARMAN, M., CHUA, A. Y. & SANTOS, G. N. C. J. H. 2019. Structures, mechanical properties and antibacterial activity of Ag/TiO2 nanocomposite materials synthesized via HVPG technique for coating application. 5**,** e01475.

MULANI, M. S., KAMBLE, E. E., KUMKAR, S. N., TAWRE, M. S. & PARDESI, K. R. J. F. I. M. 2019. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. 10**,** 539.

NAKBUBPA, K., JANCHAWNA, R., THUMCHOP, W., PANBOONTHONG, A. & PORNSUKAROM, S. 2021. Prevalence, species, and antimicrobial resistance of Acinetobacter in surgical practice and laboratory dog husbandry room environments. *Korean Journal of Veterinary Research,* 61**,** 26.1-26.8.

NATARAJAN, K. J. C. 2015. Antibiofilm activity of epoxy/Ag-TiO2 polymer nanocomposite coatings against Staphylococcus aureus and Escherichia coli. 5**,** 95-114.

NEMEC, A., DIJKSHOORN, L. & VAN DER REIJDEN, T. J. 2004. Long-term predominance of two pan-European clones among multi-resistant Acinetobacter baumannii strains in the Czech Republic. *Journal of medical microbiology,* 53**,** 147-153.

References

NEWSOM, S., PARAMESHWARAN, H. P., MARTIN, L. & RAJAN, R. 2021. The CRISPR-Cas mechanism for adaptive immunity and alternate bacterial functions fuels diverse biotechnologies. *Frontiers in cellular and infection microbiology***,** 898.

OBEROI, A., AGGARWAL, A. & LAL, M. 2009. A Decade of an Underestimated Nosocomal Pathogen-Acinetobacter In a Tertiary Care Hospital in Punjab. *JK science,* 11.

OIKONOMOU, O., SARROU, S., PAPAGIANNITSIS, C., GEORGIADOU, S., MANTZARLIS, K., ZAKYNTHINOS, E., DALEKOS, G. & PETINAKI, E. 2015. Rapid dissemination of colistin and carbapenem resistant Acinetobacter baumannii in Central Greece: mechanisms of resistance, molecular identification and epidemiological data. *BMC infectious diseases,* 15**,** 1-6.

OLIVEIRA, A. & MARIA DE LOURDES, R. J. B. R. N. 2010. Comparison of methods for the detection of biofilm production in coagulase-negative staphylococci. 3**,** 1-8.

OTOKUNEFOR, K., OGUGBUE, C. & FAJOYOMI, B. 2020. Enterobacterial repetitive intergenic consensus (ERIC) as a tool for genetic characterisation of bacterial isolates in Nigeria. *Nigerian Journal of Biotechnology,* 37**,** 122-128.

ÖZÇELIK, H. B., YILDIRIM, T., MARAKLI, S. & IDIL, Ö. J. R. I. M. M. 2020. Investigation of oxacillinases type beta-lactamases in carbapenems resistant Acinetobacter baumannii clinical isolates. 31**,** 209-214.

PAPP-WALLACE, K. M., ENDIMIANI, A., TARACILA, M. A. & BONOMO, R. A. 2011. Carbapenems: past, present, and future. *Antimicrobial agents and chemotherapy,* 55**,** 4943-4960.

PAVEL, A. B. & VASILE, C. I. 2012. PyElph-a software tool for gel images analysis and phylogenetics. *BMC bioinformatics,* 13**,** 1-6.

References

PELEG, A. Y., SEIFERT, H. & PATERSON, D. L. 2008. Acinetobacter baumannii: emergence of a successful pathogen. *Clinical microbiology reviews,* 21**,** 538-582.

PERRY, J. D. J. C. M. R. 2017. A decade of development of chromogenic culture media for clinical microbiology in an era of molecular diagnostics. 30**,** 449-479.

POIREL, L. & NORDMANN, P. 2006. Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. *Clinical Microbiology and Infection,* 12**,** 826-836.

POMPILIO, A., SCRIBANO, D., SARSHAR, M., DI BONAVENTURA, G., PALAMARA, A. T. & AMBROSI, C. 2021. Gram-Negative Bacteria Holding Together in a Biofilm: The Acinetobacter baumannii Way. *Microorganisms,* 9**,** 1353.

PROCOP, G., CHURCH, D., HALL, G., JANDA, W., KONEMAN, E., SCHRECKENBERGER, P., WOODS, G. J. P. G., CHURCH DL, HALL GS, JANDA WM, KONEMAN EW, SCHRECKENBERGER PC, WOODS GL. KONEMAN'S COLOR ATLAS & HEALTH, T. O. D. M. T. E. W. K. 2017. 12: Gram-positive cocci. 695.

RAHEEM, H. Q. & AL-HASNAWY, H. H. 2020. Molecular investigation of antibiotic resistance genes in extensive drug resistant (xdr) acinetobacter baumannii isolated from clinical specimens in babylon province, iraq. *Biochemical and Cellular Archives* 20**,** 4349-4356.

RAJKUMARI, S., PRADHAN, S., SHARMA, D. & JHA, B. 2020. Prevalence and Antibiogram of Acinetobacter Species Isolated from Various Clinical Samples in a Tertiary Care Hospital. *Journal of College of Medical Sciences-Nepal,* 16**,** 26-32.

RAMIREZ, M. S., BONOMO, R. A. & TOLMASKY, M. E. 2020. Carbapenemases: Transforming Acinetobacter baumannii into a yet more dangerous menace. *Biomolecules,* 10**,** 720.

References

RANGEL, K., CHAGAS, T. P. G. & DE-SIMONE, S. G. J. P. 2021. Acinetobacter baumannii infections in times of COVID-19 pandemic. 10**,** 1006.

RAUT, S., RIJAL, K. R., KHATIWADA, S., KARNA, S., KHANAL, R., ADHIKARI, J. & ADHIKARI, B. 2020. Trend and characteristics of Acinetobacter baumannii infections in patients attending Universal College of Medical Sciences, Bhairahawa, Western Nepal: a longitudinal study of 2018. *Infection and Drug Resistance,* 13**,** 1631.

SARI, N. D., BALTALI, S., SERIN, I., ANTAR, V. J. C. J. O. I. D. & MICROBIOLOGY, M. 2021. Evaluation of Intraventricular/Intrathecal Antimicrobial Therapy in the Treatment of Nosocomial Meningitis Caused by Multidrug-Resistant Gram-Negative Bacteria after Central Nervous System Surgery. 2021.

SARSHAR, M., BEHZADI, P., SCRIBANO, D., PALAMARA, A. T. & AMBROSI, C. 2021. Acinetobacter baumannii: An Ancient Commensal with Weapons of a Pathogen. *Pathogens,* 10**,** 387.

SEHREE, M. M., ABDULLAH, H. N. & JASIM, A. M. 2021. Isolation and Evaluation of Clinically Important Acinetobacter Baumannii From Intensive Care Unit Samples. *Journal of Techniques,* 3**,** 83-90.

SEHREE, M. M., ABDULLAH, H. N. & JASIM, A. M. 2022. Comparison of phenotypic and genotypic assays of biofilm formation in A. baumannii isolates based on gold standard method and related antibiotic resistance. *Gene Reports***,** 101575.

SHEN, M., LUAN, G., WANG, Y., CHANG, Y., ZHANG, C., YANG, J., DENG, S., LING, B., JIA, X. J. D. M. & DISEASE, I. 2016. Coexistence of blaOXA-23 with armA in quinolone-resistant Acinetobacter baumannii from a Chinese university hospital. 84**,** 230-231.

SHIELDS, P. & CATHCART, L. J. A. S. F. M. 2010. Oxidase test protocol. 1-9.

References

SINGH, H., THANGARAJ, P. & CHAKRABARTI, A. 2013. Acinetobacter baumannii: a brief account of mechanisms of multidrug resistance and current and future therapeutic management. *Journal of clinical and diagnostic research: JCDR*, 7**,** 2602.

SMANI, Y., FÀBREGA, A., ROCA, I., SÁNCHEZ-ENCINALES, V., VILA, J. & PACHÓN, J. 2014. Role of OmpA in the multidrug resistance phenotype of Acinetobacter baumannii. *Antimicrobial agents and chemotherapy*, 58**,** 1806-1808.

SMILINE GIRIJA, A. 2019. CLSI based antibiogram profile and the detection of MDR and XDR strains of Acinetobacter baumannii isolated from urine samples. *Medical journal of the Islamic Republic of Iran*, 33**,** 3.

SUBHADRA, B., KIM, D. H., WOO, K., SURENDRAN, S. & CHOI, C. H. 2018. Control of biofilm formation in healthcare: Recent advances exploiting quorum-sensing interference strategies and multidrug efflux pump inhibitors. *Materials*, 11**,** 1676.

SULTAN, A. & NABIEL, Y. 2019. Tube method and Congo red agar versus tissue culture plate method for detection of biofilm production by uropathogens isolated from midstream urine: Which one could be better? *African Journal of Clinical and Experimental Microbiology*, 20**,** 60-66.

SYKES, E. M., DEO, S. & KUMAR, A. 2020. Recent advances in genetic tools for Acinetobacter baumannii. *Frontiers in genetics***,** 1657.

TANG, B., GONG, T., ZHOU, X., LU, M., ZENG, J., PENG, X., WANG, S. & LI, Y. 2019. Deletion of cas3 gene in Streptococcus mutans affects biofilm formation and increases fluoride sensitivity. *Archives of oral biology*, 99**,** 190-197.

TEIXEIRA-SANTOS, R., GOMES, M., GOMES, L. C. & MERGULHÃO, F. J. J. I. 2021. Antimicrobial and anti-adhesive properties of carbon

nanotube-based surfaces for medical applications: a systematic review. 24**,** 102001.

TSITSOPOULOS, P. P., IOSIFIDIS, E., ANTACHOPOULOS, C., ANESTIS, D. M., KARANTANI, E., KARYOTI, A., PAPAEVANGELOU, G., KYRIAZIDIS, E., ROILIDES, E. & TSONIDIS, C. 2016. Nosocomial bloodstream infections in neurosurgery: a 10-year analysis in a center with high antimicrobial drug-resistance prevalence. *Acta neurochirurgica,* 158**,** 1647-1654.

TYUMENTSEVA, M., MIKHAYLOVA, Y., PRELOVSKAYA, A., TYUMENTSEV, A., PETROVA, L., FOMINA, V., ZAMYATIN, M., SHELENKOV, A. & AKIMKIN, V. J. P. 2021. Genomic and phenotypic analysis of multidrug-resistant Acinetobacter baumannii clinical isolates carrying different types of CRISPR/Cas systems. 10**,** 205.

VAIRO, C., BASAS, J., PASTOR, M., PALAU, M., GOMIS, X., ALMIRANTE, B., GAINZA, E., HERNANDEZ, R., IGARTUA, M. & GAVALDÀ, J. 2020. In vitro and in vivo antimicrobial activity of sodium colistimethate and amikacin-loaded nanostructured lipid carriers (NLC). *Nanomedicine: Nanotechnology, Biology and Medicine,* 29**,** 102259.

VAN BELKUM, A., SORIAGA, L. B., LAFAVE, M. C., AKELLA, S., VEYRIERAS, J.-B., BARBU, E. M., SHORTRIDGE, D., BLANC, B., HANNUM, G. & ZAMBARDI, G. 2015. Phylogenetic distribution of CRISPR-Cas systems in antibiotic-resistant Pseudomonas aeruginosa. *MBio,* 6**,** e01796-15.

VAN BELKUM, A., TASSIOS, P., DIJKSHOORN, L., HAEGGMAN, S., COOKSON, B., FRY, N., FUSSING, V., GREEN, J., FEIL, E. & GERNER-SMIDT, P. 2007. Guidelines for the validation and application of typing methods for use in bacterial epidemiology. *Clinical Microbiology and Infection,* 13**,** 1-46.

References

VANDEPITTE, J., VERHAEGEN, J., ENGBAEK, K., PIOT, P., HEUCK, C. C., ROHNER, P. & HEUCK, C. 2003. *Basic laboratory procedures in clinical bacteriology*, World Health Organization.

VÁZQUEZ-LÓPEZ, R., SOLANO-GÁLVEZ, S. G., JUÁREZ VIGNON-WHALEY, J. J., ABELLO VAAMONDE, J. A., PADRÓ ALONZO, L. A., RIVERA RESÉNDIZ, A., MULEIRO ÁLVAREZ, M., VEGA LÓPEZ, E. N., FRANYUTI-KELLY, G. & ÁLVAREZ-HERNÁNDEZ, D. A. J. A. 2020. Acinetobacter baumannii resistance: a real challenge for clinicians. 9**,** 205.

VECITIS, C. D., ZODROW, K. R., KANG, S. & ELIMELECH, M. J. A. N. 2010. Electronic-structure-dependent bacterial cytotoxicity of single-walled carbon nanotubes. 4**,** 5471-5479.

VILLALÓN, P., VALDEZATE, S., CABEZAS, T., ORTEGA, M., GARRIDO, N., VINDEL, A., MEDINA-PASCUAL, M. J. & SAEZ-NIETO, J. A. J. B. M. 2015. Endemic and epidemic Acinetobacter baumannii clones: a twelve-year study in a tertiary care hospital. 15**,** 1-9.

VRANCIANU, C. O., GHEORGHE, I., CZOBOR, I. B. & CHIFIRIUC, M. C. 2020. Antibiotic resistance profiles, molecular mechanisms and innovative treatment strategies of Acinetobacter baumannii. *Microorganisms,* 8**,** 935.

WANG, H., WANG, M., XU, X., GAO, P., XU, Z., ZHANG, Q., LI, H., YAN, A., KAO, R. Y.-T. & SUN, H. 2021. Multi-target mode of action of silver against Staphylococcus aureus endows it with capability to combat antibiotic resistance. *Nature communications,* 12**,** 1-16.

WHO 2014. *Antimicrobial resistance: global report on surveillance*, World Health Organization.

References

WILFINGER, W. W., MACKEY, K. & CHOMCZYNSKI, P. J. B. 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. 22**,** 474-481.

XIAO, J., ZHANG, C. & YE, S. 2019. Acinetobacter baumannii meningitis in children: a case series and literature review. *Infection,* 47**,** 643-649.

YAHAV, D., GISKE, C. G., GRĀMATNIECE, A., ABODAKPI, H., TAM, V. H. & LEIBOVICI, L. J. C. M. R. 2020. New β-lactam–β-lactamase inhibitor combinations. 34**,** e00115-20.

YANG, C.-H., SU, P.-W., MOI, S.-H. & CHUANG, L.-Y. J. M. 2019. Biofilm formation in Acinetobacter Baumannii: genotype-phenotype correlation. 24**,** 1849.

YAZDANI, M. R., SHEIKHPOUR, M., SIADAT, S. D. & SAFARIAN, P. 2021. Overcoming the antibiotic resistance of Acinetobacter baumannii by using nanofluid containing functionalized carbon nanotubes. *Nanomedicine Research Journal,* 6**,** 179-187.

YOON, E.-J., COURVALIN, P. & GRILLOT-COURVALIN, C. 2013. RND-type efflux pumps in multidrug-resistant clinical isolates of Acinetobacter baumannii: major role for AdeABC overexpression and AdeRS mutations. *Antimicrobial agents and chemotherapy,* 57**,** 2989-2995.

YU, W.-C., WANG, T., ZHANG, G.-Q., WANG, Z.-G., YIN, H.-M., YAN, D.-X., XU, J.-Z., LI, Z.-M. J. C. S. & TECHNOLOGY 2018. Largely enhanced mechanical property of segregated carbon nanotube/poly (vinylidene fluoride) composites with high electromagnetic interference shielding performance. 167**,** 260-267.

ZEIGHAMI, H., VALADKHANI, F., SHAPOURI, R., SAMADI, E. & HAGHI, F. J. B. I. D. 2019. Virulence characteristics of multidrug resistant biofilm forming Acinetobacter baumannii isolated from intensive care unit patients. 19**,** 1-9.

References

ZHANG, Z., MORGAN, C. E., BONOMO, R. A. & YU, E. W. J. M. 2021. Cryo-EM determination of Eravacycline-Bound structures of the Ribosome and the multidrug efflux pump AdeJ of Acinetobacter baumannii. 12**,** e01031-21.

ZOWAWI, H. M., SARTOR, A. L., SIDJABAT, H. E., BALKHY, H. H., WALSH, T. R., AL JOHANI, S. M., ALJINDAN, R. Y., ALFARESI, M., IBRAHIM, E. & AL-JARDANI, A. 2015. Molecular epidemiology of carbapenem-resistant Acinetobacter baumannii isolates in the Gulf Cooperation Council States: dominance of OXA-23-type producers. *Journal of clinical microbiology,* 53**,** 896-903.

Appendix

bioMérieux Customer:
System #:

Laboratory Report

Printed Nov 6, 2020 06:42 CST
Printed by: Labadmin

Patient Name:
Isolate: 0611212-1 (Qualified)

Patient ID:

Card Type: GN Bar Code: 2411530403259014 Testing Instrument: 00000A726B5A (AL-NUKHBA LAB)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0201010103500212
Organism Quantity: **Selected Organism: Acinetobacter baumannii**

Comments:	

Identification Information	Card: GN	Lot Number: 2411530403	Expires:	Feb 3, 2022 12:00 CST
	Completed: Nov 5, 2020 14:08 CST	Status: Final	Analysis Time:	5.80 hours
Organism Origin	VITEK 2			
Selected Organism	99% Probability Acinetobacter baumannii Bionumber: 0201010103500212		Confidence:	Excellent identification
SRF Organism				

Analysis Organisms and Tests to Separate:
Acinetobacter baumannii

Acinetobacter baumannii 44C(99),41C(99),

Analysis Messages:

Contraindicating Typical Biopattern(s)

ALNOKHBA LAB

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

bioMérieux Customer:
System #:

Laboratory Report

Printed Nov 6, 2020 06:42 CST
Printed by: Labadmin

Patient Name:
Isolate: 0611212-1 (Qualified)

Patient ID:

Card Type: GN Bar Code: 2411530403259014 Testing Instrument: 00000A726B5A (AL-NUKHBA LAB)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0201010103500212
Organism Quantity:

Selected Organism: Acinetobacter baumannii

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	+	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	+			

ALNOKHBA LAB

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

ALNUKHBAH LABORATORY مختبر النخبة

bioMérieux Customer:
System #:

Laboratory Report

Printed Nov 6, 2020 06:43 CST
Printed by: Labadmin

Patient Name:
Isolate: 0611211-1 (Qualified)

Patient ID:

Card Type: GN Bar Code: 2411530403259013 Testing Instrument: 00000A726B5A (AL-NUKHBA LAB)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0201010201500250
Organism Quantity:

Selected Organism: Acinetobacter baumannii

Comments:	

Identification Information	**Card:** GN		**Lot Number:** 2411530403	**Expires:**	Feb 3, 2022 12:00 CST
	Completed: Nov 5, 2020 13:10 CST		**Status:** Final	**Analysis Time:**	4.83 hours
Organism Origin	VITEK 2				
Selected Organism	99% Probability	**Acinetobacter baumannii**			
	Bionumber: 0201010201500250			**Confidence:**	Excellent identification
SRF Organism					

Analysis Organisms and Tests to Separate:

Acinetobacter baumannii

Acinetobacter baumannii	44C(99),41C(99),

Analysis Messages:

Contraindicating Typical Biopattern(s)

ALNOKHBA LAB

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

bioMérieux Customer:
System #:

Laboratory Report

Printed Nov 6, 2020 06:43 CST
Printed by: Labadmin

Patient Name:
Isolate: 0611211-1 (Qualified)

Patient ID:

Card Type: GN Bar Code: 2411530403259013 Testing Instrument: 00000A726B5A (AL-NUKHBA LAB)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0201010201500250
Organism Quantity:

Selected Organism: Acinetobacter baumannii

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	+	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	-	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	+	62	ELLM	-	64	ILATa	-			

مختبر النخبة
ALNOKHBA LAB

Installed ViTEK 2 Systems Version: 08.01
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

الخلاصة

تعد *A. baumannii* من مسببات الأمراض الانتهازية السالبة لصبغ الجرام، والتي تسبب التهابات شديدة ويصعب علاجها. تم جمع (150) عينة سريرية من مرضى يعانون من التهابات مختلفة. وتم تنمية جميع العينات السريرية على وسط الدم وسط MacConkey و CHROMagar ثم تم تأكيد تشخيص بكتريا *A. baumannii* عن طريق الاختبارات البيوكيميائية وجهاز vitek 2 بعدها تم إجراء اختبار الحساسية للمضادات الحيوية بطريقة انتشار القرص القياسية لعشرة مضادات مختلفة. بعدها تم قياس قدرة تكوين الغشاء الحيوي بواسطة البكتريا بطريقة لوحة زراعة الأنسجة.

علاوة على ذلك، تم فحص تأثير $AgTIO_2$ و SWCN-OH ضد نمو عزلات *A. baumannii* والغشاء الحيوي بشكل منفصل عن طريق إجراء خمسة تخفيفات متسلسلة مزدوجة عند (2/1 ، 4/1 ، 8/1 ، 16/1 و 32/1) تركيز المحلول الاصلي (تركيز 1000 ميكروغرام / ملـــ).

للوصول إلى وجود الجين *cas* ، تم أولاً استخلاص الحمض النووي من جميع عزلات *A. baumann*ii وتم إجراء تفاعل البوليميراز المتسلسل المتعدد لتضخيم ستة جينات مختلفة. اما بالنسبة للتنوع الجيني، تم اجراء BOX-PCR و ERIC-PCR و تحليل النتائج المستحصلة باستخدام PyElph الإصدار 1.4. تم إنشاء مخططات dendrograms باستخدام طريقة مجموعة الزوج غير الموزونة بمتوسط حسابي (UPGMA).

أظهرت النتائج ان ثلاثة وعشرون عينة تابعة لبكتريا وبنسبة (15.3٪) من العدد الكلي للعينات ، و كالتالي 12 عزلة (26.6٪) من البلغم ، وخمس عزلات (14.2٪) من الحروق ، وثلاث عزلات (7.5٪) من الجروح وثلاثة (10٪) من البول. وجميع هذه العزلات كانت مقاومة لـ Cefepime (100٪) بينما في حالة مضادات الميكروبات الأخرى ظهرت النتائج التالية (91٪) مقاومة إلى Trimethoprim-Sulfamethoxazole، (80٪) Gentamicin ، Amikacin، (77٪) Ciproloxacin ، Ceftazidime ،Levofloxacin، (73٪) إلى Amoxicillin-clavulanica acid، و (70٪) Meropenem ، بينما تنخفض نسبة المقاومة (50٪) Tetracyclin.

في هذه الدراسة جميع عزلات *A.baumannii* قادرة على تكوين بيوفيلم قوي. لذلك ، تم اختبار تأثير $AgTIO_2$ والأنابيب النانوية الكربونية أحادية الجدار SWCN-OH على نموها والغشاء الحيوي ، وأظهرت النتائج أن هناك انخفاضًا كبيرًا في نمو البكتيريا والغشاء الحيوي بعد التعرض

للتخفيف التسلسلي المزدوج لـ $AgTIO_2$ الأنابيب النانوية الكربونية الأحادية الجدار، لكن كانت الفعالية الاكبر بواسطة SWCN-OH.

أظهرت نتائج التحليلات الجينية التي تهدف إلى الكشف عن جينات *cas* لـ *A. baumannii* أن جميع العينات المدروسة تحتوي على هذا الجين على النحو التالي ، تم العثور على الجين*Cas1* بحجم 506 bp و 196 bp *cas2* و *cas3* 850 bp في 5 (25٪) ، 3 (15٪) ، 9 (45٪) من العزلات على التوالي. تم اكتشاف الجين 5 *cas* في 3 (15٪) من العزلات ، والجين 6 *cas* في 7 (35٪) من العزلات ، وأخيراً الجين 9 *cas* في 6 (30٪) من العزلات.

في حالة النوع I-F CRISPR-Cas جين (الذي ينتمي إلى نظام النوع الأول) ، تم تحديد هذا الجين (157 bp) في 7 من اصل 20 عينة من العينات المتنوعة وعلى النحو التالي: 3 (100٪) بول ، 2 (22.22٪) بلغم ، 1 (20٪) حروق و 1 (33.3٪) جروح.

اما بالنسبة لاكتشاف التنوع الوراثي للBOX و ERIC-PCR في *A. baumannii* المعزولة من عينات مختلفة ، بشكل عام ، كانت لعزلات جميعها مميزة باستخدام الطريقتين الجزيئيتين.و أنتجت التقنيتان الجزيئيتان مجموعتان رئيسيتان ونتائج مخطط الشجرة لهذه التقنيات تكشف أن 20 جميع العزلات متعدد الأشكال بين عزلات *A. baumannii* السريرية تم اكتشافها بواسطة ERIC و BOX PCR.

في الختام، تُظهر البيانات ارتباطًا بين عوامل الضراوة وتكوين الأغشية الحيوية بين سلالات *A. baumannii* العراقية المتعددة المقاومة للمضادات الحياتية وتساعد هذه البيانات على فهم المقاومة البكتيرية وبقائها في المستشفيات العراقية. بالإضافة إلى فعالية الجسيمات النانوية التي قد تساعد في المستقبل على التحكم في الانتشار بعد اجراء الدراسات التكميلية التجريبية الأخرى.

وزارة التعليم العالي
والبحث العلمي
جامعة بابل
كلية الطب

التنوع الوراثي وتوصيف أنظمة CRISPR-Cas لعزلات *Acinetobacter baumannii* المتعددة المقاومة للمضادات الحياتية

رسالة

مقدمة الى مجلس كلية الطب / جامعة بابل

كجزء من متطلبات نيل درجة الماجستير في علم الأحياء المجهرية الطبية

من قبل

نادية عبد العباس كاظم بيرم

بكالوريوس علوم حياة/ كلية العلوم جامعة بابل

2001

بأشراف

الاستاذ

د.ميساء صالح مهدي الشكري

١٤٤٣ هـ ٢٠٢٢ م