A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF ÇANKIRI KARATEKİN UNIVERSITY

EFFECT OF ZINC OXIDE NANOPARTICLES USE AS ALTERNATIVE TREATMENT FOR *PSEUDOMONAS AERUGINOSA*

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGY

BY

HIBA ADNAN ABDULHUSSEIN

ÇANKIRI

2022

EFFECT OF ZINC OXIDE NANOPARTICLES USE AS ALTERNATIVE TREATMENT FOR *PSEUDOMONAS AERUGINOSA*

By Hiba Adnan ABDULHUSSEIN

April 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

Advisor : Asst. Prof. Dr. Yaşar Kemal YAZGAN
Co-Advisor : Asst. Prof. Dr. Mohanad W. Mahdi ALZUBAIDY

Examining Committee Members:

Chairman : Assoc. Prof. Dr. Oral DÜZDEMİR
Department of Biology
Çankırı Karatekin University

Member : Asst. Prof. Dr. Yaşar Kemal YAZGAN
Faculty of Dentistry
Çankırı Karatekin University

Member : Asst. Prof. Dr. Nilifer CİHANGİR
Department of Biology
Hacettepe University

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. İbrahim ÇİFTÇİ
Director of Graduate School

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Hiba Adnan ABDULHUSSEIN

ABSTRACT

EFFECT OF ZINC OXIDE NANOPARTICLES USE AS ALTERNATIVE TREATMENT FOR *PSEUDOMONAS AERUGINOSA*

Hiba Adnan ABDULHUSSEIN

Master of Science in Biology

Advisor: Asst. Prof. Dr. Yaşar Kemal YAZGAN

Co-Advisor: Asst. Prof. Dr. Mohanad W. Mahdi ALZUBAIDY

April 2022

The current study included collection of 150 samples from Burns and Wounds during the period from October 2021 to January 2022. Patients with different ages ranging from 16-70 years from both genders were choosing from different hospitals in Baqubah city/Diyala Province, Iraq included Baqubah Teaching Hospital and Al-Batool Teaching Hospital. After culturing the specimens on general culture media, 121 of it were positive growth for bacteria. Pseudomonas agar, microscopic investigation, biochemical checks and VITEK 2 Compact were used to isolate Pseudomonas aeruginosa which consist 25 (16.6 %), 16 isolates were from Burns and 9 isolates were from wounds. The anti-biogram of the isolates was tested against 10 antibiotics which were selected according to (CLSI 2021). The result showed that Cefepime and polymyxin B were the most resisted by the bacteria (100%), while Ciprofloxacin and Norfloxacin were the greatest in effect antibiotics in contrast to P. Aeruginosa (12% and 16%) respectively. The ZnO NPs preparation was done by sol-gel technique and it was detected by (FTIR), (XRD) and Field (FE-SEM). The anti-bacterial action of ZnO NPs in contrast to P. Aeruginosa was conducted at dissimilar concentrations (100 µg/mL, 50 µg/mL, 25 µg/mL and 12.5 µg/mL). Which were examined in contrast to PDR, XDR and MDR isolates, the effects showed that the highest inhibition area of isolates was at concentration 100 µg/mL and the lowermost inhibition area was at concentration 12.5 µg/mL. The minimum inhibition concentration of the Zno NPs was at a concentrations ranging from 2600µg/mL -162.5µg/mL on PDR, XDR and MDR isolates of the bacterium P. Aeruginosa.

2022, 79 pages

Keywords: *Pseudomonas Aeruginosa*, Zinc oxide, Isolation,

ÖZET

PSEUDOMONAS AERUGINOSA İÇİN ALTERNATİF TEDAVİ OLARAK KULLANILAN ÇİNKO OKSİT NANOPARÇACIKLARININ ETKİSİ

Hiba Adnan ABDULHUSSEIN

Biyoloji, Yüksek Lisans

Tez Danışmanı: Dr. Öğr. Üyesi Yaşar Kemal YAZGAN

Eş Danışman: Dr. Öğr. Üyesi Mohanad W. Mahdi ALZUBAIDY

Nisan 2022

Mevcut çalışma Ekim 2021'den Ocak 2022'ye kadar olan dönemde yanık ve yaralardan toplanan 150 numunenin üzerinde gerçekleştirilmiştir. Her iki cinsiyetten de yaşları 16-70 arasında değişen hastalar, Irak'ın Diyala Eyaletindeki Bakubah şehrindeki Bakubah Eğitim Hastanesi ve Al-Batool Eğitim Hastanesi'ni de içeren farklı hastanelerden seçilmişlerdir. Numuneler kültür ortamında kültürlendikten sonra 121 tanesinde bakteri üremesi pozitif olmuştur. Pseudomonas agar, mikroskobik inceleme, biyokimyasal kontroller ve VITEK 2 Compact kullanılarak 25 izolat (%16,6) Pseudomonas aeruginosa'dan, 16 izolat yanıklardan ve 9 izolat yaralardan izole edilmiştir. İzolatların antibiyogramları, (CLSI 2021)'e göre seçilen 10 antibiyotiğe karşı test edilmiştir. Sonuç olarak, en fazla bakterinin direnç gösterdiği (%100) Cefepime ve polimyxin B iken, *P. Aeruginosa*'ya karşı en etkili antibiyotiklerin Ciprofloxacin(%12) ve Norfloxacin(%16) olduğu tespit edilmiştir. ZnO NP'lerin hazırlanması sol-jel tekniği ile yapılmış ve (FTIR), (XRD) ve Field (FE-SEM) ile tespit edilmiştir. Farklı konsantrasyonlardaki ZnO NP'lerin (100 µg/mL, 50µg/mL, 25 µg/mL ve 12,5 µg/mL) P. Aeruginosa'nın PDR, XDR ve MDR izolatlarına karşı antibakteriyel etkileri incelenmiş, izolatların en yüksek inhibisyon alanının 100 µg/mL konsantrasyonunda olduğu ve en düşük inhibisyon alanının 12.5 µg/mL konsantrasyonunda olduğu gözlemlenmiştir. Zno Np'lerin minimum inhibisyon alanı, *P. Aeruginosa* bakterisinin PDR, XDR ve MDR izolatlarında 2600 µg / mL -162,5µg / mL arasında değişen konsantrasyonlarda meydana gelmiştir.

2022, 79 sayfa

Anahtar Kelimeler: *Pseudomonas Aeruginosa*, Çinko oksit, İzolasyon,

PREFACE AND ACKNOWLEDGEMENTS

First of all, praise and thanks be to God alone (Glory be to Him), for the shower of blessings throughout the period of my dissertation for successfully completing the thesis. I would like to extend my thanks, respect and appreciation to my advisor, Asst. Prof. Dr. YAŞAR KEMAL YAZGAN and Co-Advisor Asst. Prof. Dr. Mohanad W. Mahdi ALZUBAIDY. I would also like to thank the Turkish people and Çankırı Karatekin University, teachers and administrators alike, for their support.

I would like to express my thanks and gratitude to my dear father and mother who are my eternal support. I will not be able to pay their rights, and my love for them cannot be described in words. I feel the need to thank my dear husband who put so much effort into supporting me by creating the right atmosphere for my studies. Thanks to my dear brothers and sisters.

Hiba Adnan ABDULHUSSEIN

Çankırı-2022

CONTENTS

LIST OF SYMBOLS

%	Percentage
^{0}C	Centigrade
g	Gram
L	Liter
mL	Milliliter
µg	Miligram
µL	Microliter

LIST OF ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
AK	Amikacin
ATM	Aztreonam
BHI	Brain heart infusion
CDC	Central of diseas control
CF	Cystic fibrosis
CIP	Cipfloxacin
CLSI	Clinical & laboratory standards ınstitute
CPM	Cefepime
EPS	Exo-poly saccharide
FDA	Food drug administration
FE-SEM	Field emission electron microscopy
FTIR	Fourier transformed infra-red spectroscop
GM	Gentamicin
GRAS	Generally recognized as safe
HIV	Human immunodeficiency virus
ICU	Intensive care unit
IMI	Imipenem
LPS	Lipopolysaccharide
MDR	Multidrug resistance
MHA	Mueller hinton agar
MIC	Minimum inhibitory concentration
NA	Nutrient agar
NB	Nutrient broth
NIH	National institute of health
NOR	Norfloxacin
OM	Outer membrane
PB	Polymyxin B
PDR	Pan drug resistance
PTZ	Piperacilline/tazobactam
ROS	Reactive oxygen species
T3SS	Type 3 secretion system
TBSA	Total body surface area
TN	Tobramycin
WHO	World health organization
XDR	Extensive drug resistance
XRD	X Ray diffraction
ZnONPs	Zinc oxide nanoparticles

LIST OF FIGURES

LIST OF TABLES

1 INTRODUCTION

Pseudomonas aeruginosa is consider as an omnipresent G-negative aerobic bacteria, an opportunistic pathogen broadly spread and causes nosocomial infections (Al-Mayali and Salman 2020) in addition to deadly infections in immune-compromised humans especially those patients with malignancy, post-surgery, burns that could be severe or infested with human immune-deficiency virus (HIV) (Gomila *et al*. 2018) despite the advent of newer and stronger ant-ibiotics (Amoon *et al.* 2018).

The strains of *P. aeruginosa*, especially multi-drug-resistant, which give rise to difficult complications in many countries, including Iraq. The rising spread of nosocomial cases formed by multidrug-resistant (MDR), extensively drug resistant (XDR) and pan-drug-resistant (PDR) *P. aeruginosa* strains poses a threat for anti-microbial treatment (El Zowalaty *et al.* 2015).

These bacteria cause a number of types of infection which include wounds, gastrointestinal, urinary tract, and respiratory tract infections. The infections by these bacteria are increasing worldwide due to the persistence, adaptation, and resistance mechanisms of altered types of anti-microbials (Abdallah and Gabur 2021). Also, infection with *P. aeruginosa* is predominantly grim to treat as a result of many intrinsic and acquired tools of antibiotics resistance (Wijaya 2021).

World Health Organization categorized *P. aeruginosa* as one of the serious pathogens in its earliest issued out group of anti-biotic unaffected priority pathogens grounded on the urgency of necessity for different anti-biotics (WHO 2017, Willyard 2017).

Nanoparticles of metal oxides having a size range of 1–100 nm represent a new orientation that is increasingly being progressed for use in research and medically-care related implementation (Anbuvannan *et al.* 2015). ZnO NPs have a large attention due to its inexpensive production cost, harmless and can be prepared without difficulty (Jayaseelan *et al.* 2014). It has a extensive range of bio-medical uses like drug carriage,

anti-malignancy, anti-diabetic, anti-bacterial, anti-fungal and agricultural properties (Kaur *et al.* 2015). A Little is identified about the anti-bacterial action of ZnO as nanoparticles (Jones *et al.* 2008). In addition, ZnO is one of five zinc compounds that are presently registered as general recognized as safely by the World Health Organization (WHO) (Lee *et al.* 2017).

Aim of the study

The current study aims to explore the antibacterial action of Zinc Oxide Nanoparticles (ZnO NPs) against multidrug-resistant bacteria (MDR), extensively drug resistant bacteria (XDR) and pan-drug-resistant bacteria (PDR) isolates of *Pseudomonas aeruginosa* by the following steps:

1) Isolation and diagnosis of *P. aeruginosa* from clinical specimens.

2) Detection of some virulence factors for the isolates.

3) Investigations the occurrence of multi-drug resistant and antibiotic susceptibility profile in *Pseudomonas aeruginosa* isolates.

4) Investigations the anti-bacterial action of Zno NPs against MDR, XDR and PDR isolates of *P. aeruginosa*, in addition to the minimum inhibitory concentration (MIC) for the ZnO NPs.

2 LITERATURE REVIEW

2.1 Common Features of *Pseudomonas Aeruginosa*

Pseudomonas aeruginosa is G-negative bacillus and motile existing nearly everywhere in the environment such as in soil, water, plants, animals, and humans, it is considered as one of the main nosocomial pathogens which feature a high percentage of patient morbidity and mortality (Klockgether and Tümmler 2017)

Pseudomonas aeruginosa is consider an opportunistic bacterium that infects immune-compromised or hospitalized patients preferably and causes more or less serious infections that may lead to death, from local to systemic. There are limited nutritional requirements for this pathogen and it can adapt to a wide range of environmental conditions. Because of the high propensity of this pathogen to establish resistance, treatment of *P. aeruginosa* infections with antibiotics has also been difficult, thereby contributing to its pathogenicity. Several healthcare organizations have identified multi-drug resistant isolates of *P. aeruginosa* as significant threat to public health. This pathogen has been described as a crucial research target for the production of innovative therapies, given its high prevalence with associated high mortality rates and restricted treatment options (Tacconelli 2017, WHO 2017).

Pseudomonas aeruginosa strains are usually motile via a singular polar flagellum. The optimum growth temperature of *P. aeruginosa* is 37°C, while its extreme growth temperature is 42°C (Brooks *et al.* 2016). Bacterial colonies appear cloudiness through the semi-solid media (around the stabbing area), which is an evidence of the motility of *P. aeruginosa* by its polar flagella (Moore *et al*. 2006). *P. aeruginosa* is an aerobic bacillus that can be considered an opportunistic pathogen. It is a highly flexible microorganism capable of tolerating conditions of low oxygen with low nutrient levels and it can live and grow at temperatures ranging from 4-42°C (Stover *et al.* 2000). *P. aeruginosa* produces pigments that inhibit the growth of bacteria of other kinds of bacteria. The most significant pigments are Pyocyanin, a water-soluble blue pigment,

and Pyoverdin, a green-yellowish pigment also known as Pseudobactin, which is toxic to the host cells (Orlandi *et al*. 2015).

This bacterium can produce various pigments, comprising the bluish-green Pyocyanin and yellowish-green fluorescein, along with the potential for other pigments, such as yellow Pyoverdin, dark Pyorubin, and dark black Pyomelanin, to be produced by certain strains (Cohen *et al.* 2016). Occasionally *P. aeruginosa* strains produce only Pyoverdin, which is difficult to distinguish these strains from the other five fluorescent Pseudomonas species (Luis *et al.* 2020). *P. aeruginosa* possesses a mucous layer to produce the alginate slime layer which inhibits the process of phagocytosis. In addition to multi-layers of extra-cellular polysaccharides (Al-Daraghi and Al-Badrwi 2020). Pseudomonas has slight nutritional supplies for their growth as a group and able to custom a extensive range of environmental nutrition sources, *P. aeruginosa* frequently requires acetate and ammonia which consider a basis of carbon and nitrogen, respectively. Also, *P. aeruginosa* is capable of anaerobic growth and does not ferment, but rather obtains energy from sugar oxidation. In environments with low concentrations of nutrients such as dry-surfaces of the hospital operating rooms, hospital rooms, health center, and medical apparatus, along with sinks and showers, this slight nutritional requirement supports it to grow and consequently has proven to be an important source of nosocomial infection (Farahi *et al.* 2018).

In many countries, including Iraq, *P. aeruginosa* isolates, particularly multidrug-resistant, have initiated grim complications. The growing occurrence of nosocomial multidrug-resistant (MDR) infections, extensively drug-resistant (XDR) and pan drug-resistant (PDR) of *P. aeruginosa* strains poses a big challenge to anti-microbial remedy (El Zowalaty *et al*. 2015). Due to its high conflict to various groups of anti-biotics and its capability to cause severe health-related as well as nosocomial infections, *P. aeruginosa* is consider one of the greatest significant bacterial species for public health reasons (Nielsen 2015).

2.1.1 Pathogenicity

P. aeruginosa is an opportunistic human pathogen, especially in patients who are immunocompromised (Al-Mayali and Salman 2020), often fatal infections (Palavutitotai *et al.* 2018), and can also cause plant disease (Schroth *et al.* 2018). It deems one of the most causative agents of nosocomial contagions in Baghdad (Al-Shimmary *et al.* 2017).

As a nosocomial bacterium, *P. aeruginosa* has the ability to colonize wounds and catheters and thus can be promoted among various hospital sectors such as urology units, burns units, and intensive care units (ICUs) (Al-Saeedi and Raheema 2019). Urinary tract infections, primary skin infections (burn and wound with blue-green pus), eye infection, ear infection, soft tissue infections, intra-abdominal infections (Tang *et al.* 2017), bacteremia (Hilliam *et al.* 2020), and cystic fibrosis (CF) that caused by patients with lung infections which consider the dominant lung infecting organism (O'Toole 2018), was communal infections resulted from this bacterium (Al-Dahmoshi 2017). *P. aeruginosa* is capable of invading tissues and producing toxins that lead to complex infections, colonizes the mucous membrane and skin, and thus causes infections that are stimulated by pili and then by the growth of virulent factors such as toxins and enzymes such as protease, which break down protein fibers to reveal bacterial receptors, and its ability to attack tissues that rest on its conflict to phago-cytosis and the host immune defenses and its excretion of external enzymes and toxins such as protease, elastase, coagulase, hemolysin, lipase, gelatinase, DNase, as well as alkaline phosphatase, lecithinase which are external virulence agents that break down physical barriers and participate in invasion bacterial (Al-Mamari 2019). Its infection efficacy and antibiotic resistance capacity have made the organism recognized as a public health threat (Golle *et al.* 2017). Where *P. aeruginosa* has recently been announced by the World Health Organization to be one of the main concern pathogens for which new anti-biotics are desperately desired (WHO 2019).

In intensive care units (ICU), *P. aeruginosa* ranks among the most dreaded of the top five microbial causing thermal burns, pulmonary, blood-stream, urinary tract,

postsurgical wounds, septicemia and soft-tissue infections (Veesenmeyer *et al.* 2009). It was besides the 2nd prominent reason of nosocomial pneumonia (14 ~ 16%), the 3rd utmost public reason of urinary tract infections (7 ~ 11%), the 4th utmost recurrently isolated pathogen in surgical place infections (8%), and the 7th primary contributor to infections of blood-streams 2~6% (Lister *et al.* 2009).

2.1.2 Epidemiology

P. aeruginosa is extensive spread microorganism in natural habitations. It can be isolated from different environmental places like plants, soil, water, animals and humans (Kordes *et al.* 2019). However, it is rarely isolated from sea water (except the water outfalls from sewage and polluted river estuaries) (Schroth *et al.* 2018).

Approximately, any kind of hospital instrument or tool has been implicated as a closet for *P. aeruginosa*, including, antiseptics, disinfectants, eyewash solutions, and intravenous fluids. These sources may serve as an epicenter for the spreading of the organism in common origin outbreaks, and this frequently is the result of poor sterilization (Brooks *et al*. 2016).

Many Iraq's studies have shown that the frequency of *P. aeruginosa* in burn contagions had a high ratio, as it ranged between 32-66% in provinces of Karbala, Kirkuk, Hilla and Baghdad, with the highest percentage recorded in Baghdad, Karbala, Hilla and Kirkuk (Al-Saadi 2009, Al-Khafaji 2016, Khorsheed andAl Abdeen 2017). Also, *P. aeruginosa* isolated from wound infections recorded as highest percentage (23.5%) among the other bacterial genera isolated in Baghdad Province (Mun *et al*. 2009). In Tikrit city, *P. aeruginosa* was recorded the highest rate in middle ear infections reached up to 42% (Kamal *et al.* 2018).

The direct and indirect contact between patients, and using the polluted surgical tools assists the spreading of these organism**.** Whereas, the bacterium possesses the capability

to survive in dis-infectants, treatments and fluid medications, such as eye drops and masks that used for anesthesia (Al-Daraghi and Al-Badrwi 2020).

2.1.3 Virulence factors

Various types of virulence factors (Ullah *et al.* 2017) are released by *P. aeruginosa*, such as hemolysin, lipopolysaccharide, flagellum, type IV pili, quorum sensing, pigments, siderophores, and a group of enzymes such as proteases, elastase, sialidases (or called neuraminidase), Dnase, gelatinase, as well as several toxins such as exotoxin A and the type III secretion system (T3SS) toxins like exotoxin U, exotoxin Y, exotoxin T, and exotoxin S (Mahdavi *et al.* 2017). These factors enable bacteria to make and withstand infections or diseases in various host hosts and tissues (Yeboah 2021). These factors are major virulence factors that affect the immune system in different ways, and these factors damage the immune system of their host and represent a barrier to antibiotics that reduce the effectiveness of antibiotics, leading to ineffective and failure treatments

2.1.3.1 Hemolysis

It is a potential virulence agent produced by bacteria, which could endanger human health. It causes lysis of red blood cells by disrupting the cell membrane, and hemolysin is believed to be in charge for various events in the host cell. Since red blood cells (RBCs) consider gorgeous in iron-containing heme, the degradation of RBCs discharges heme in the surrounding medium, which allows the bacteria to absorb free iron. Hemolysin is thereason for tissue damage, assisting bacterial proliferation, the release of host nutrients, and may moreover adapt host signaling pathways that affect many manners, which include host cell survival, inflammatory responses, and cyto-skeletal dynamic. Most *P. aeruginosa* produces beta-hemolysin, which is the entire blood lysis. Enzyme virulence factors that damage tissue include hemolysin (Reda *et al.* 2017).

2.1.3.2 Pigment production

P. aeruginosa yields various pigments. The utmost main of which is the blue-green pigment of Pyocyanin pigment, which is observed on the surface of the cultivated petri-dish and is mentioned to as the blue pus, Pyomelanin pigment is black and pyorubin pigment is red (Riedel *et al.* 2022).

Pyocyanin is a product that consider the secondary metabolism. It belongs to the family of phenazine because it has the nucleus of phenazine, and in addition to being a virulence agent. It acts as a bio-sensor signal molecule, participating in a diversity of vital activities which include gene expression, and it keeps the vitality of the generating germ cells. It supplies the foundation of the bio-film and is distinguished by its antibacterial and antifungal activity and cause oxidative damage to tissues, in particular to oxygenated tissues such as the lung and it participates in oxidative stress that promotes the alteration of the host's mitochondrial electron transport (Aykac *et al.* 2017).

2.1.3.3 Biofilm formation

Bio-films are populations of micro-organisms adhering to the surface that could be biotic or not biotic and enclosed by a matrix of exo-polysaccharide (EPS) or as microbial groups living in a self-produced matrix principally be made of poly-saccharides, extra-cellular DNA, and proteins (Ciofu and Tolker-Nielsen 2019). It is a common cause of chronic infection and is regulated by QS systems (Mukherjee *et al.* 2018). It is normal that biofilms show an environmental role and have a major influence in medicine through the development of healthcare-associated infections. Biofilm formation by *P. aeruginosa* is one of the main causes of therapeutic failure and increases morbidity and mortality through its protection against the host's immune system and antibiotic therapy. It is estimated to be involved in 65 % of infectious diseases and more than 80 % of bacterial infections caused by the National Institutes of Health (NIH) (Jamal *et al.* 2018).

The main stage in the production of biofilms is the synthesis of the extracellular matrix. It contains all components except for bacterial cells. Matrix is the main structural feature of bacterial biofilm by containing up to 90 % of total organic matter. It is strongly hydrated and is mainly composed of exopolysaccharides, proteins, nucleic acids, and minerals. Their composition depends on the type of bacteria and the conditions of growth. Helps to strengthen the structure of biofilms while maintaining high elasticity. It also plays a defensive function as it increases the tolerance of bacteria to antimicrobials by forming a physical barrier that prevents their spread to other environmental factors (pH, UV rays, changes in osmotic pressure, dehydration) and tolerance mechanisms, for example, contributes to distinct extra-cellular matrix or an-aerobic environments (Brauner *et al.* 2017, Trastoy *et al.* 2018). Hence, when *P. aeruginosa* is presented in stress circumstances, biofilm formation is frequently related to higher anti-microbial resistance compared to the planktonic form and helps to avoid the host's immune response (Skariyachan *et al.* 2018).

P. aeruginosa infection associated with the development of biofilms is extra common in immune-compromised patients and patients who have medical devices implantation in the lungs and middle ear, in addition to in patients with contact lenses, catheters, and other implants. The development of these bacteria may be asymptomatic inside the human body till the bacteria form a bio-film that overcomes the immune-system. These biofilms could be lethal in patients with cystic fibrosis and primary ciliary dyskinesia (Gerrard *et al.* 2016).

2.2 Antibiotics

Antibiotics are therapeutic agents that used in modern healthcare due to its powerful activity in fighting of life-threatening infections. Antibiotics are defined as natural organic substances with the ability to inhibit/kill the other competitive organisms. It is produced by some particular microorganisms at low concentrations. A long time ago, human tried to find treatments of microbial infections. Dyes, molds, and also heavy metals were thought to be used for healing. The practice of anti-microbial steward-ship

revolves about the concept of optimizing anti-microbial healing and decreasing adverse events through economically accountable means (Cunha *et al*. 2018).

2.2.1 Mechanism of action of antibiotics

Mechanisms of action of antibiotic on the bacterial cell can be divided into five categories (Etebu and Arikekpar 2016, Chaudhary *et al*. 2017):

- Cell wall synthesis inhibition (Penicillins e.g. Ticarcillin)
- Protein synthesis inhibition (Aminoglycosides e.g. Amikacin)
- Nucleic acid synthesis inhibition (Fluoroquinolones e.g. Levofloxacin)
- Folate synthesis inhibition (Trimethoprim)
- Disruption structures of the cytoplasmic membrane (Daptomycin)

2.2.2 Anti-biotic resistance of *P. aeruginosa*

Anti-biotic resistance can be defined as the ability of microbes to defeat the antimicrobial agents intended to kill them. Recently, antibiotics resistance is the greatest global public health challenges. In 2017, the World Health Organization (WHO) published a list of 12 genera and/or families, prioritized for the development of alternative antimicrobials. They categorized them into critical, high, and medium. Carbapenems-resistant genera were classified as one of the main priority critical group of antimicrobial resistance of clinical, environmental, and control strains of *P. aeruginosa* (Shrivastava *et al.* 2018).

Unfortunately, nowadays, many people around the world are dying due to the untreatable infections caused by the development and break-out of anti-biotic resistance. In addition, the antibiotics resistance causes significant economic loss to the health care centers and hospitals of the high cost of treatment and the long duration of the patient's stay in hospital (Yusuf *et al*. 2017, Ullah *et al*. 2017).

The methods of intrinsic and acquired anti-biotic resistance of *P. aeruginosa* due to only restricted number of anti-biotics are persist unclear in handling of infections that caused by this bacteria. The disproportionate usage of anti-biotics through handling quickens the development of multi-drug resistant in *P. aeruginosa* strains. This lead to an unsuccessfulness of the use anti-biotic healing against this micro-organism (Esmaeili *et al*. 2019).

In 2013, the Center for Disease Control (CDC) was published a table of anti-biotic resistance dangers and then modernized the table in 2019. The table was ordered in a levels of “urgent threats”, “serious threats” and "concerning threats". The serious threats are those antibiotics that requirement checking as they have the capability to become impending threats to the public health. Those Gram-negative bacteria which involved multi-drug resistant of *P. aeruginosa*. The high percentage in morbidity and mortality in affected patients is because the elevating in the antibiotic resistance in immunocompromised patients and healthcare-associated with this pathogen (Rocha *et al.* 2019, McCutcheon *et al.* 2018). Based on the drug resistance patterns of the organism, *P. aeruginosa* is divided into different phenotypes. These are included multi-drug resistant (MDR), extensively-drug resistant (XDR) and pan-drug resistant (PDR) (Golle *et al*. 2017).

1) Multi-drug resistant (MDR) as being acquired non-sensitive to at minimum 1 anti-biotic in 3 or more anti-microbial groups (Hawkey *et al.* 2018). The MDR of *P. aeruginosa* is related to increase morbidity and mortality and delaying hospital stay.
2) Extensive-drug resistance (XDR) is defined as non-sensitive bacteria to at minimum 1 agent in all but 2 or less anti-microbial groups (i.e. bacterial isolates persist susceptible to only 1 or 2 groups) (Hawkey *et al.* 2018).
3) Pan-drug resistant (PDR) is well-defined as non-sensitive to all anti-biotics in all anti-microbial groups (Hawkey *et al.* 2018).

To confirm the accurate application of these explanations, bacterial isolates had better to be tried against totally or almost all of the anti-microbial agents within the anti-

microbial groups (Magiorakos *et al.* 2012). The pan-drug resistance, the deficiency of antibiotics effectiveness in contrast to MDR *P. aeruginosa* isolates and remains the dissemination of such strain result in serious challenges in the infection control administration (Murugan *et al.* 2018).

2.2.3 Mechanism of antibiotic resistance in *Pseudomonas aeruginosa*

The tools resistance are defense approaches developed by microbes to help them survive and avoid the effects of antibiotics. *P. aeruginosa* has turn into an imperative and recurrent opportunistic nosocomial pathogen. This organism is categorized by a native resistance to multiple categories of anti-microbials which initiating difficulty in treating infections which could be lead to high morbidity and mortality (Hassuna *et al*. 2020). As well known, *P. aeruginosa* is difficult to control by agents like antibiotics and antiseptics.

The extensive use of antibiotics to treat the infections caused by this bacterium has led to an emerge of strains with multiple antibiotic resistance increased over time, making it one of the furthermost shared health complications, specifically for those patients remaining in hospitals (Dantas and Sommer 2014). *P. aeruginosa* related resistance is frequently can be classified into intrinsic/native resistance and acquired or modified resistance (Blair *et al.* 2015). Figure 2.1 summarizes the types of mechanisms of resistance in *P. aeruginosa.*

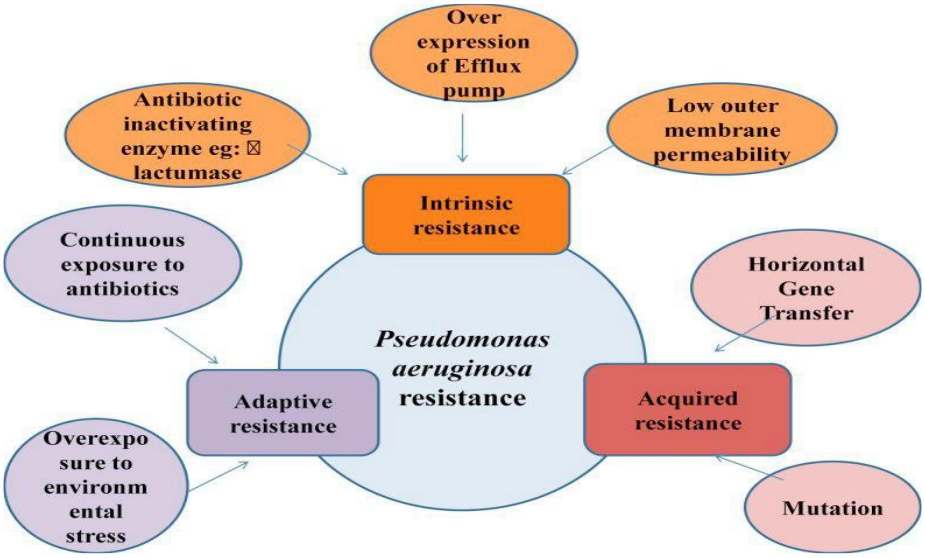


Figure 2.1 *P. aeruginosa* resistance by diiferent mechanisms (Pachori *et al.* 2019)

2.2.3.1 Intrinsic resistance

P. aeruginosa contain remarkable intrinsic apparatuses of resistance and is capable of obtaining numerous mechanisms of anti-biotic resistance, displays inherent resistance to anti-microbial agents by a diversity of mechanisms: (1) reduced permeability of the outer-membrane, (2) efflux systems that actively push anti-biotics out of the cell, and (3) creation of anti-biotic incapacitating enzymes (Moore and Flaws 2011).

(1) Outer membrane permeability

The outer membrane (OM) of *P. aeruginosa* principally feats as a penetrability barrier and reports a extensive spectrum of intrinsic anti-biotic resistance (Purro *et al.* 2018). The outer membrane (OM) is an asymmetric bi-layer comprising of lipo-poly-saccharides (LPS) in the outer leaf-let and phospholipids in the interior leaf-let, non-specific porins, and definite up-take channels are embedded.

(2) Efflux systems

Among the mechanical resistance that the bacteria use in contrast to anti-biotics, efflux pump activity increasing, which mechanism by improving the efflux of anti-biotics from the bacterial cell, is one of the greatest noticeable and thus a potential object of mixture remedy and can be ordered into 5 classes: resistance-nodulation-division (RND) family, major facilitator superfamily (MFS), ATP-binding cassette (ABC) superfamily, small multidrug resistance (SMR) family, and multidrug and toxic compound extrusion (MATE) family (Cepas and Soto 2020).

(3) Antimicrobial inactivating enzymes

It is consider one of the focal tools of intrinsic resistance that used by the bacteria is the formation of anti-biotic incapacitating enzymes that disruption down or adapt anti-biotics, which is the hydro-lytic deactivation of the beta-lactam ring in Penicillins and Cephalosporins by a bacterial enzyme termed beta-lactamase. Beta-lactamases are enzymes that covalently bind to the lactam ring, hydrolyze it, and render the antibiotic ineffective (Torok *et al.* 2009). In this group, Beta-lactamases, Chloramphenicol, Aminoglycosides, and Erythromycin-modifying enzymes are the most common examples (Sharkey and O'Neill 2019).

2.2.3.2 Acquired resistance

Acquired resistance happens when a specific micro-organism obtains the capability to resist an anti-microbial agent or because it is not affected by the antibiotics to which it was previously susceptible. Acquired resistance comes from major chromosome or extra-chromosomal structures (plasmids, transposons, etc.) (Aljanaby and Aljanaby 2018).

Chromosomal resistance results from mutations that randomly change the bacterial chromosome. These mutations can be triggered by certain physical and chemical factors (Majeed and Aljanaby 2019). Extra-chromosomal resistance depends on extra-chromosomal genetic material that can be transmitted via plasmids, transposons, and

integrons (Jabuk *et al.* 2017). Usually, the plasmid is responsible for developing inactive antibiotic enzymes (Witwit *et al.* 2019).

2.2.3.3 Adaptive resistance

Adaptive resistance discusses the resistance that happens because of the environmental circumstances, like transcriptional alterations in genes that regulate resistance/susceptibility, and rise the capability of a bacterium to persist under an anti-biotic dose due to transient changes in gene and/or proteins expression in reply to an environmental stimulus and is reversible when environmental circumstances (for example, complex adaptive growth states such as swarming, bio-film formation, or exposure to stresses, involving antibiotics) are reversed or can be reversed when the stimulus is gone, finally adaptive resistance that is a reflection of the environmental status of the bacterium and includes genetic changes caused by the environment (Schroeder *et al.* 2017, Pang *et al.* 2019).

2.3 Nanoparticles NPs

The word "Nano" is unoriginal from Latin, meaning dwarf. The mass of the nano refers to one thousand millionth of a specific units, hence, nano-meter means one thousand millionth of a meter (i.e. 1nm = 10-9 m) (Gleiter 2000). Nano-technology can be definite by Professor Norio Taniguchi in 1974, as a multi-disciplinary science that shields a various area of research and technology in chemistry, physics and biology (Uskokovic 2008). Nano-technology is consider an imperative field of recent research trade with synthesis, strategy and manipulation of particle structure ranging from nearly 1 to 100 nm in size. Within this size range all the features (chemical, physical and biological) variations in both individual atoms/molecules and their corresponding bulk are investigated (Ghanbarzadeh *et al.* 2015). Nano-particles are commonly definite as those things that contain one or extra external dimensions in the nano-scale (1 to 100 nm), that exhibit new or enhanced size-dependent properties compared to the bigger particles of the similar material (Shah and Shah 2013). Unique uses of nano-particles are growing quickly on numerous fronts because of their improved properties based on

size, distribution and morphology. It is gaining renovation in a huge number of fields like health care, cosmetics, bio-medical, food and feed, drug-gene delivery, environment, optics, chemical industries, electronics, energy science, catalysis, light emitters, single electron transistors and photo-electrochemical uses (Ghanbarzadeh *et al.* 2015, Madhumitha *et al.* 2016).

The exact machineries of anti-bacterial activity of NPs are not completely understood. Nevertheless, various studies proposed the following mechanisms of anti-bacterial effects of NPs. First, NPs can attach to the bacterial cell membrane by electro-static interaction and able to disruption the integrity of bacterial membrane. Bacterial cell wall is aimed to provide inflexibility, strength, and shape, and defend the cell from burst and mechanical damage (De Azeredo 2013). The structure and configuration of bacterial cell wall can be categorized into two kinds: Gram positive bacteria and Gram negative bacteria. The configuration of the cell wall shows a crucial role in the outcome of NPs on bacteria Figure 2.2. Properties of bacterial cell wall plays a key role in distribution of NPs inside the bio-film matrixes (Baek and An 2011).

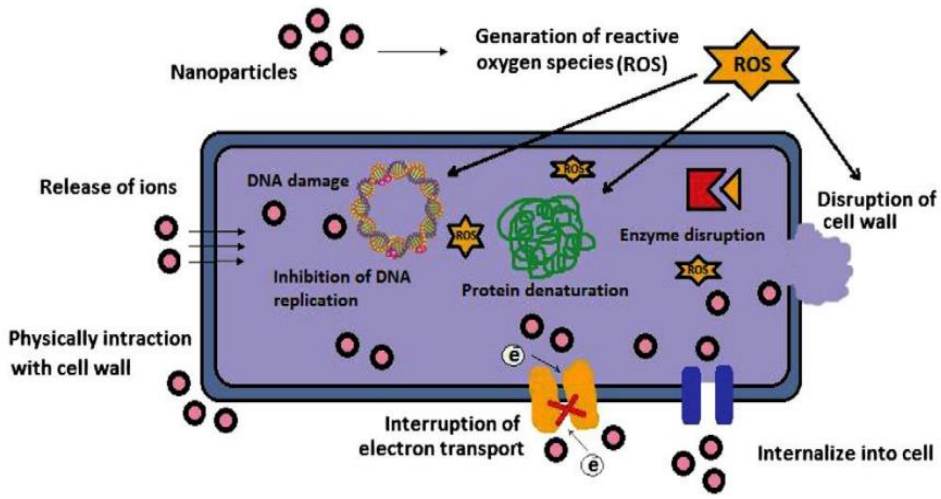


Figure 2.2 Antimicrobial activity mechanism of the nanoparticles

2.3.1 Properties of nanoparticles

Nanoparticles are very important in the mathematical fields due to their properties and its unique features (Sahayaraj and Rajesh 2011):

1) It has a small size ranges between 1-100 nanometers.

2) It has a very large surface area compared to its size.

3) It has distinct biological and chemical properties.

4) The structural rigidity these particles possess despite their small atomic structure.

5) The assemblies of enhanced nanoparticles depend on the nature of the surface change of the optical signals to which the substance is exposed.

6) High temperature endurance, electrical conductivity ability, enhanced surface activity catalyst.

2.3.2 Synthesis of nanoparticles

Nanoparticles synthesis occurred through two approaches: Bottom-Up and Top-Down show as Figure 2.3

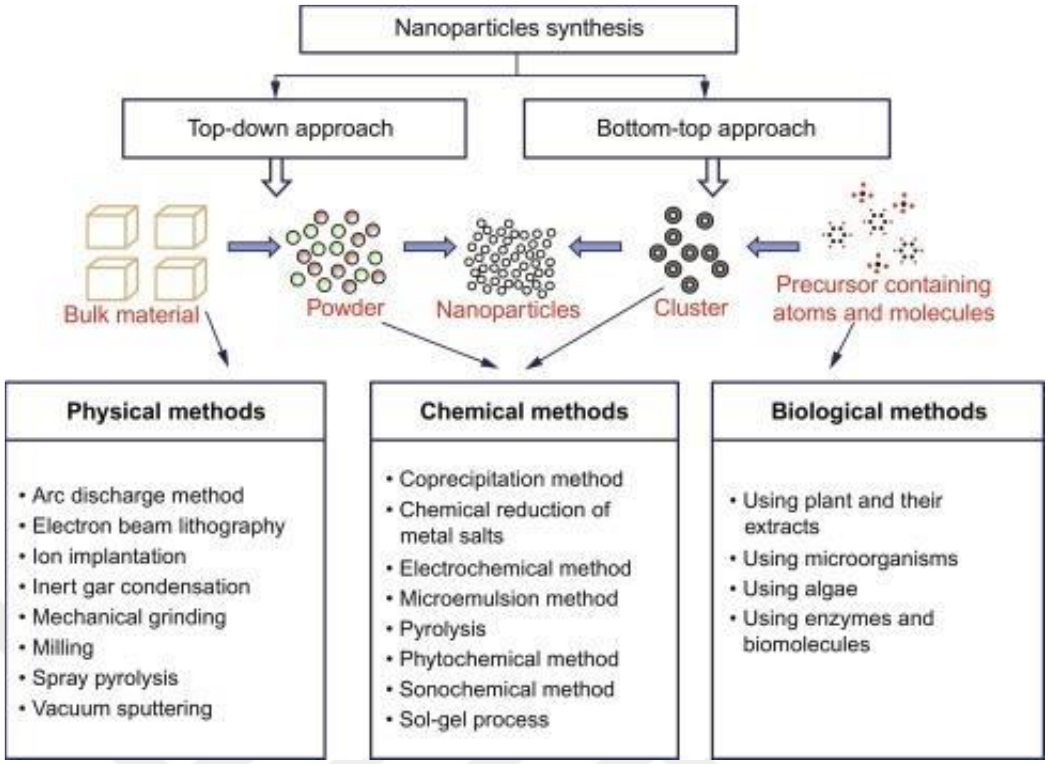


Figure 2.3 Methods of Nano Particls synthesis

2.3.3 Zinc oxide nanoparticles ZnO NPs

Zinc oxide is consider a type of semi-conducting metal oxide. It has a extensive range of applicability in the electronics, optics, and bio-medical systems (Anbuvannan *et al.* 2015). Numerous kinds of inorganic metal oxides have been synthesized and continued in recent studies like TiO_2, CuO, and ZnO. Among these metal oxides, ZnO NPs are consider the maximum interest because they are low-cost to yield, harmless and can be synthesized easily (Jayaseelan *et al.* 2014). US-Food and Drug Administration (FDA) has registered the ZnO as GRAS (generally recognized as safe) metal oxide and also can be consumed as food additive as in the fortification of cereal-based foods (Sabir *et al.* 2014). ZnO NPs exhibit ultra-violet filtering characters, anti-inflammatory and wound curative (Mirzaei and Darroudi 2017). It has a extensive range of bio-medical applications like drug delivery, anti-cancer, anti-diabetic, anti-bacterial, anti-fungal and agricultural characters (Jain *et al.* 2014). Although ZnO can be used for directed drug delivery, it still has the limitation of cytotoxicity which is until now have to be resolved (Ma *et al.* 2013). Bio-synthesis ZnO NPs have a very robust anti-bacterial influence at a

little concentration of gram negative and gram positive bacteria as established by the studies. They have displayed robust anti-bacterial influence than the ZnO NPs synthesized chemically (Vimala *et al.* 2014, Madhumitha *et al.* 2016). ZnO NPs have been reported in different morphological structure such as nano-flake, nano-flower, nano-belt, nano-rod and nano-wire, to be utilized as antimicrobial agents to reduce multidrug resistant bacteria (Elkady *et al.* 2015, Agarwal *et al.* 2017).

3 MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipments and apparatus

The equipments and apparatus that were used in this study are shown in Table 3.1

Table 3.1 Equipment and apparatus

Equipments and apparatus	Company	Origin
Autoclave	GEMMY	Taiwan
Baker	Sigma	USA
Centrifuge	Thermo Scientific™	USA
Disperser	MAST	UK
Disposable Petri-dishes	Grenier	Germany
Distillator	Gallenkamp	England
ELISA reader	Human	Germany
ELSIA washer	Human	Germany
Field Emission Scanning Electron Microscope (FE-SEM)	Shemadzu	Japan
Flask	Sigma	USA
Hot plate with magnetic sterror	Memmert	Germany
Incubator	Memmert	Germany
Inoculating loop	Eppendorf	Germany
Laminar air flow	LabTech	Korea
Light Microscope	Olympus	Japan
Magnetic bar	Shemadzu	Japan
Microcentrifuge	Eppendorf	Germany
Micropipettes of different sizes	Human	Germany
Microplate reader	Human	Germany
Microwave Oven	GOSONIC	China
Parafilm	Jenway	Germany
pH meter	Hanna	Italy
Refrigerator	TEKA	Spain
Sensitive balance	OHAUS- PioNEER	USA
Shaker incubator	Sartorius	Germany
Spectrophotometer Fourier Transform Infra-Red (FTIR)	Shemadzu	Japan
Sterilized cotton swabs	Sterellin Ltd.	UK
Vortex	Quality Lab system	England
Water bath	Memmert	Germany
Well flat bottom microplate	Coastar	USA

3.1.2 Biological and chemical materials

Chemical and biological materials that were used in current study are mentioned in Table 3.2

Table 3.2 Biological and chemical materials

Chemical and biological material	Company	origin
Absolute ethanol	Romil	France
Amonia	SCR	Canada
Catalase solution: Hydrogen peroxide 3% (H_2O_2)	Analar	England
Citric acid	Sigma	USA
Glycerol	Fluka	Switzerland
Oxidase reagent: Oxidase indicator [N,N,N, N-tetramethyl-p-phenylenediamine dihydrochloride%1]	Himedia/	India
Potassium bromide	Sigma	USA
Standard McFarland's solution (0.5)	BioMérieux	France
Zn (CH3CO2)2 2H2o	Sigma	USA

3.1.3 Culture media

Culture media that used in current study mentioned in the Table 3.3 are prepared according to instructions of manufacturing company. Briefly, the powder was dissolved in distilled water and heated by hot plate with continue stirring to dissolve all the ingredients entirely then sterilized by autoclave. The sterilized media were poured in sterile Petri dishes. The plates were incubated for 24 hours at 37 °C to confirm their sterility after being prepared. They saved at 4°C until they used (Forbes *et al.*, 2007).

Table 3.3 Culture media

Media	Company	origin
Blood agar	MAST	UK
Brain heart infusion broth (BHIB)	MAST	UK
Muller Hinton agar (MHA)	Oxoid	UK
Nutrient agar	Himedia	India
Nutrient broth (NB)	Himedia	India
MacConkey agar	Himedia	India
Pseudomonas Agar Base with CFC supplement	MAST	UK

3.1.4 Antibiotics

Antibiotic disks (Mast group Ltd. /UK) were used are listed in Table 3.4 according to (CLSI 2021)

Table 3.4 Antibiotics

Antibiotic class	Antibiotic type	Antibiotic symbol	Concentration mg/disc
ß-lactam combination agent	Piperacillin/Tazobactam	PTZ	100/10
Cephalosporins	Cefepime	CPM	30
Fluoroquinolones	Ciprofloxacin	CIP	5
	Norfloxacin	NOR	10
Aminoglycosides	Gentamicin	GM	10
	Amikacin	AK	30
	Tobramycin	TN	10
Monobactams	Aztreonam	ATM	30
Carbapenems	Imipenem	IMI	10
Lipopeptides	Polymyxin B	PB	300

3.2 Methods

3.2.1 Ethical consideration

A valid consent was obtained from hospital administrators. Also, all participants and health care providers were informed about the purpose of the study. Benefits were discussed; Patients were learned that the study posed no risks to their health. Participation in the study was voluntary and that participants have the correct to withdraw at any time without giving any reason and without affecting their care or health. Measures were taken to ensure confidentiality through coding the data, and participants were informed that data collected will be used only for the purpose of the study, and oral agreement was got.

3.2.2 Study design

The steps of the current study illustrated by the following diagram, Figure 3.1

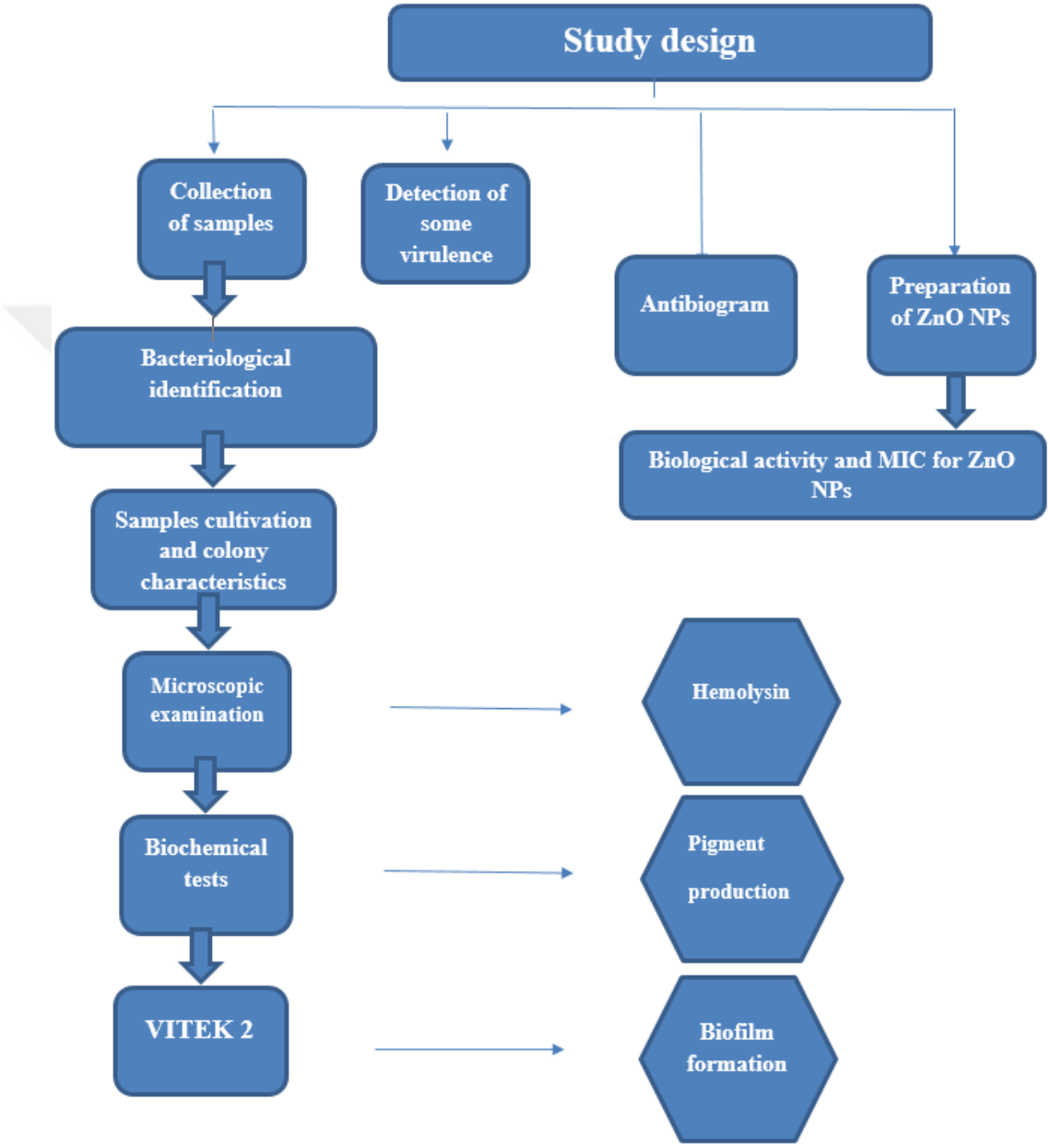


Figure 3.1 Study design

3.2.3 Sterilizations methods

Sterilization by autoclave: The media were sterilized by the moist heat sterilization method by autoclave for 15 minutes at 121℃ and pressure 15bar/in2 (James *et al.* 2020).

Sterilization by hot air oven: All the glassware was sterilized and dried by thermal oven at 180 ℃ for one hour (James *et al.* 2020).

3.2.4 Preparation of culture media

The whole culture media was prepared according to the manufactured company that used for culturing and diagnosing the bacteria (James *et al.* 2020).

3.2.5 Preparation of stains and solutions

3.2.5.1 Gram stain

The ready-made kit was used, which is a crystal violet stain, Lugol's iodine stain, decolorization solvent, and 0.5% standard safranine stain as counter-stain. The gram stains method is considered the most common method used in the microbiology laboratory, which forms the basis for the classification of bacteria into two groups (for example, gram-positive and gram-negative) (Murray *et al.* 2021).

3.2.5.2 MacFarland standard solution

To calibrate the number of bacterial cells, a prepared solution from the French company Bio Mérieux was used to give an approximate number of bacterial cells equal to 1.5×10^{8}CFU/mL (James *et al.* 2020).

3.2.5.3 Bacterial suspension

Bacterial suspensions of each isolate were prepared by transferring a single colony grown on MacConkey agar medium to 5 mL of distilled water to get a suspension with a turbidity of (1.5×10^8) CFU / mL by compared with MacFarland standard (Forbes *et al.*, 2007).

3.2.5.4 Catalase

A commercial hydrogen peroxide 3% (H_2O_2) was prepared from 15% stock solution and kept in dark tube (Forbes *et al.*, 2007).

3.2.5.5 Oxidase

Oxidase reagent preparation was done by dissolving 1g of N-N-N-N–tetramethyl-P-phenylene diamine hydrochloride in 100 mL of distilled water and stored in dark container in the refrigerator and used within a week (Forbes *et al.*, 2007).

3.2.6 Preparation of Zinc oxide nanoparticles by sol-gel method

The Zinc oxide nanoparticles (ZnO NPs) was set by sol-gel method which is mentioned by (Osman and Mustafa 2015) the basic steps are as below Figure 3.2

1) Zinc acetate di-hydrate 0.1M Zn $(CH_3CO_2)2\ 2H_2O$ and citric acid 0.1M $C_6H_8O_7$ were prepared, the Zinc acetate dihydrate was weighted in a deionized water in a beaker measuring 100 mL and then placed in a magnetic stirrer for 450 rpm at room temperature.

2) Citric acid was dissolved in another beaker by 100 mL in deionized water.

3) The dissolved substance from the citric acid was added to the dissolved substance from the zinc acetate dihydrate gradually and by continuous stirring with a magnetic stirrer.

4) The pH of the resulting solution was measured and the pH was adjusted to 7 by adding ammonia drops.

5) The heat in the magnetic stirrer was operated at 80 C / 450 rpm, the rotation continuous until a white sol-gel is consist

6) The consisted sol-gel was autoclaved at 80 C for two hrs. where then a white powder is consist.

7) The white powder was placed in an oven at 450 C for two and half hours, which at the end, white- black material is consist which is the zinc oxide nanoparticles.

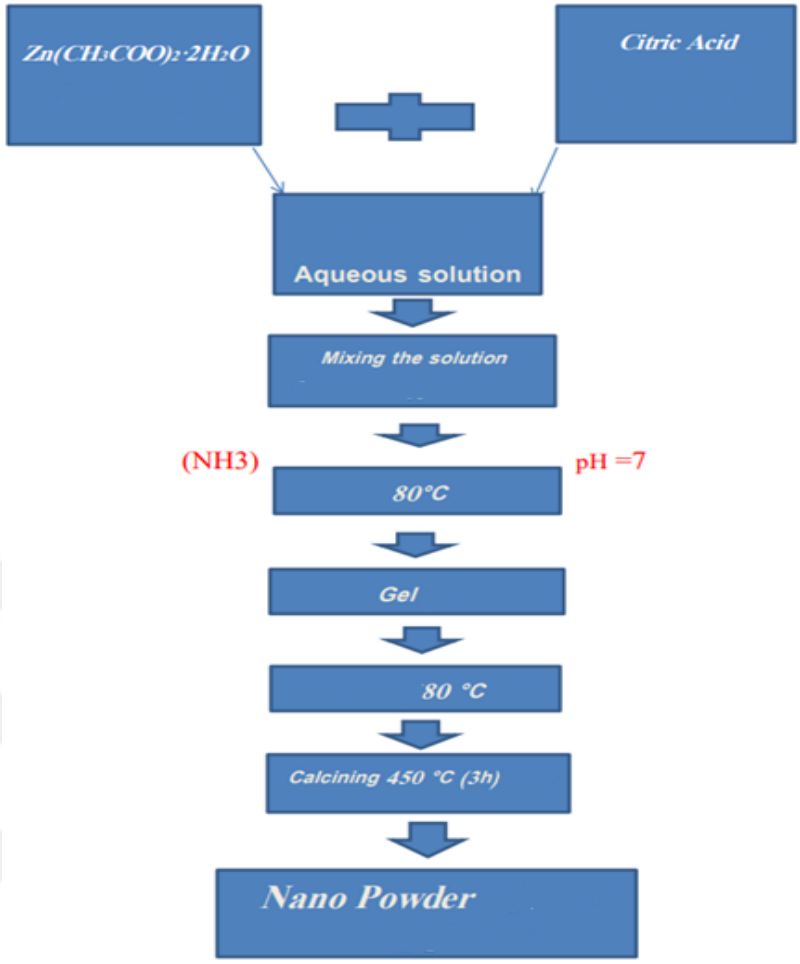


Figure 3.2 Zinc Oxide Nano Particls preparation by sol-gel method

3.2.6.1 Fourier transformed infra-red spectroscopy (FTIR)

The Fourier Transformed Infra-Red spectroscopy (FTIR) to the Zno NPs was measured by using the Fourier Transformed Infra-Red spectroscopy (FTIR) device in Diyala University/ college of pure science/ department of chemistry by taking 1 mg of Zno NPs and mixed with potassium bromide (1gram) in a ceramic mortar until a transparent tablet is consist and then it placed in the device.

3.2.6.2 X-Ray diffraction

X-ray diffraction examination was performed in university of Baghdad/ ibn al haytham to measure its crystal structure, to describe crystalline materials and for information on preferred structures, phases, crystal orientations (texture) and other structural information such as average of the granule size, crystallization, crystal defects and X-ray diffraction pattern.

3.2.6.3 Crystallite size

The crystallite size was measured by using Debye Scherrer formula (Khodair *et al.* 2019)

$D = K\lambda / \beta \cos\theta$

As:

(D) = average of crystal size, (K) = fixed amount of the used device, (λ) = X-ray wavelength (1.5416 Å), (β) =Full width at half maximum ,(Cos θ) = cosine theta X-ray diffraction

3.2.6.4 Field emission electron scanning microscope (FE-SEM)

Examinations were carried out for Zno NPs prepared by sol-gel to describe the phenotypic and surface characteristics of by using Field Emission Electron Scanning Microscope (FE-SEM) at university of Kashan / Iran, the device works on imaging the sample with am magnification 25-250000 X and scan the sample with high definition, the concentrated package interacts with the tested material in a way of electrons that collide with the sample atoms and transmit signals explain surface structure where a detector converts the concentration of the electrons into a numerical voltage and

configuring a three dimensional image, the size can be measured by this device 1-5 nanometer.

3.2.7 Collection of specimens

An overall of 150 clinical cases were collected beginning from October 2021 to January 2022. Patients with different ages ranging from 16-70 years from both genders were choosing from different hospitals in Baqubah city/Diyala Province, Iraq included Baqubah Teaching Hospital and Al-Batool Teaching Hospital. The clinical specimens were collected from wounds (70) and burns (80) using cotton disposable swabs and transported into the sterile medium in plastic bottles. All the swabs and urine containers of 150 specimens were taken under sterile conditions and transferred, inoculated immediately on MacConkey agar and blood agar and then the plate were incubated at 37 °C for 18 or 24 hrs. After that, pure growth colonies were cultured on Pseudomonas agar. The biochemical examination was carried out for identification diagnosis and characterization according to the standard routine techniques. These tests are summaries below:

3.2.8 Phenotypic identification

3.2.8.1 Morphological examination

The initially indicative checks were based on the morphological features of the bacterial growth on Pseudomonas agar and MacConkey agar. These characters were comprising colony form, colony consistency, color and edges, odor and the color of the produced pigment (Baron *et al.* 2007).

3.2.8.2 Microscopic examination

The microscopic examination of the cells of the developing bacterial isolates was carried out by transferring a portion of a young colony by the sterile inoculating loop

and mixed with a 60 micro-liter of distilled water on the surface of a clean slide, then spread on the surface of the slide, left to dry and fixed by heat on the Bunsen burner, then stained in a gram staining method and examined in a multipart light micro-scope (100x) to note the shape of the cells and the nature of their interaction with gram stain (Procop *et al.* 2017).

3.2.8.3 Biochemical tests

According to (Procop *et al*. 2016) biochemical tests were used to diagnose bacterial isolates

- **Catalase (slide test)**

Catalase development by *P. aeruginosa* was determined by transforming the middle of a 24 hrs. good growth unpolluted culture colony with a disinfected inoculating needle, then mixing it with a 60 mL of 3% hydrogen peroxide (H_2O_2) reagent and depositing it on a clean slide. The presence of fast bubbles (gas O_2) indicates a positive result for this test, while bacteria produced no catalase reaction, indicating a negative result (Kasprowicz *et al.* 2018).

- **Oxidase**

The N, N, N, N-tetramethyl-p-phenylene-diamine di-hydrochloride ready reagent powder was dissolved in 3 mL of sterile distilled water or saline and immediately placed in a dark jar to detect cytochrome C in particular for gram-negative bacteria (Hemraj *et al.* 2013).

3.2.8.4 Growth on pseudomonas agar

Pseudomonas agar as a selective medium for *P. aeruginosa* bacterial isolates. These isolates were cultured on this media and then it was incubated at 37°C for 24 hrs, blue-

green pigment indicated the presence of pyocyanin as a positive result (James *et al.* 2020).

3.2.9 Identification of the isolates by VITEK2

In this study, the purpose if using VITEK-2 system was for diagnostic conformation of *P. auruginosa* (HernándezDurán *et al.*, 2017), with an excellent grade of accuracy and this apparatus contain 64 bio-chemical checks which used for bacterial diagnosis with an precision of up to (98%) and the outcome of the investigation takes almost 8 h or fewer, as well as a test of bacterial susceptibility to anti-biotics which consisting of several stages as follows:

I- Preparation of microbial suspension

II-Inoculation of diagnostic card

III- Card sealing and incubation

3.2.10 Preservation of isolates

Two methods were used for the preservation of isolates

3.2.10.1 Preservation for a short period

The bacterial isolates were injected after being identified on the slant nutrient agar, and incubated at 37 °C for 24 hours, and stored for one to a maximum of 3 months at 4°C in the refrigerator (Fugelsang and Edwards 2007)

3.2.10.2 Preservation for a long period

The bacterial isolates of *P. aeruginosa* were put in storage for a extended period (up to 3 months) on a brain heart infusion broth medium containing 15% glycerol at -20°C

(deep freezing). The medium consisted of 2 mL of glycerol and 8 mL of brain heart infusion broth (concluding volume is 10 mL) and then autoclaved. After cooling, the medium was injected with a pure bacterial isolated colony and incubated at 37°C for 24 hours. The tubes were stored at -20°C in deep freezing (Vandepitte *et al.* 2003)

3.2.11 Detection of virulence factors of *P. aeruginosa* isolates

3.2.11.1 Hemolysin production

It was used to detect the production of haemolysin enzyme. The isolates were inoculated on the blood agar base containing 5% human blood and incubated at 37°C for 24 hrs. After incubation, total red blood cell lysis (clear area) consisted around the cultured colonies that showed hemolysin positivity (βhemolysis) (Najnin *et al.* 2018).

3.2.11.2 Pigments production

Pigment production was examined using Pseudomonas agar and Nutrient agar medium, inoculated with bacterial isolates and keep warm at 37°C for 24 hrs to visual analysis colony morphology and pigments production (Nader *et al.* 2017).

3.2.11.3 Biofilm formation

The formation of the bio-film was detected by micro-titer plate technique according to (Almeida *et al.* 2013). The bacteria were inoculated on Nutrient broth at 37°C for 24 hrs. Thereafter, the broth cultures were compared with a MacFarland standard No. 0.5 consuming the identical medium as the diluent. 200 µL of the isolate suspension were moved into each of 3 wells of the 96-wells flat-bottomed poly-styrene plate and keep warm in the incubator at 37°C for 24 hrs. Later, every well was raising 3 times with distilled water with smooth shaking and later dehydrated carefully. The sticking bacterial cells were immovable by 200 µL of absolute methanol. Next that, every well was marked with 200 µL of 0.5% crystal violet for 15 minutes. To get clear of the remaining discoloration, frequent washing was done. With 200 l of ethanol per well, the crystal violet bound to adhering cells was maintained. The test was carried out three times, with the absorbance of wells filled with bacteria-free Nutrient broth serving as a negative control. The amount of crystal violet destroyed by 95 percent ethanol in each well was determined by measuring the OD 630 nm with an ELISA reader, as described in the protocol. (Tang *et al.* 2011). Because of this, the absorbance values represented the intensity of the biofilm formed by well-studied isolates on the surface of the micro-titer. The results obtained were categorized into three groups (i.e., Non-biofilm producer, moderately, and strongly biofilm producer). Where the absorption of the

cultivated pit was compared with the control pits, as follows: if OD ≤ ODc (Considered non-biofilm producer); if ODc ≤ OD ≤ 2* ODc (Considered moderately biofilm producer); if 2* ODc ≤ OD (Considered strongly biofilm producer). Where OD (Represent the tested isolates); ODc (Represent control pits).

3.2.12 Antibiogram

3.2.12.1 Antibacterial activity of antibiotics

The sensitivity test procedure was done according to (CLSI 2021) as the following steps:

1. Mueller-Hinton agar plates were used for the rapidly growing species in the Kirby-Bauer process. The solvent was poured in the petri dishes and had a deepness of around 4 mm.

2. Uncontaminated culture has been used as inoculum; (2-4) related colonies have been transported to about 5 mL of standard sterile saline. To get an average quantity identical to (1.5x 10^8) cell/mL, the turbidity of bacterial suspension was compared with the turbidity of the McFarland Standard.

3. The sterile cotton swab was immersed into the standard inoculum, streaking was performed 3 times on the whole agar surface of the petri-dish with the swab, rotary the petri-dish between each line at 50 degrees. The inoculum had been allowed to dry with a lid in place for 5-10 minutes. 4. Using disk dispenser (MAST™), antibacterial disks were applied on MHA (Oxoid™).

5. The petri-dishes were subsequently incubated at 37 C and analyzed 18-24 h. Inhibition zones were measured, and the zones' diameters were reported to the nearest millimeter (Moreno *et al.* 2006).

3.2.12.2 Biological activity of ZnO NPs against *P. aeruginosa*

Determination of Zinc Oxide Nanoparticles antibacterial activity was done by agar well diffusion assay according to (Obedat *et al.* 2012) with some modification. Antibacterial activity was determined against multi-drug resistance bacteria

1) The first stock of ZnO NPs 100% was set by dissolved 500 mg of NPs powder in 5 mL of deionized water (the dissolving process was done by using vortex and heating the solution 40C in water bath to make sure that all the powder was dissolved completely).

2) Four different concentrations were prepared from the stock; 12,500; 25.000; 50.000; 100.000 µL/mL

3) Each bacterium was culture on Mueller Hinton agar after comparison with McFarland tube ($1.5 * 10^8$) CFU/mL by streaking method, the wells (5mm) were made in the plate by sterilized cork borer.

4) The 4 dissimilar concentrations of the ZnO NPs were add to wells by 100 µL, the fifth well represent the negative control by adding 100 µL of deionized water.

5) Three duplications for each isolate was made and then incubate at 37C for 24 hours.

6) The inhibition zone of each concentration of the Zno NPs was determined by a standard ruler in millimeters.

3.2.13 Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) can be defined as the lowermost concentration of ZnO NPs that's inhibits the growth of the bacteria. Broth dilution technique was done to quantify the MIC. A stock of ZnO NPs, suspension was made at a concentration of 1µg/µL (1 mg of NPs were dissolved in 1 mL of distilled water). Suitable concentrations of ZnO NPs were made conferring to their working manual, using Mueller Hinton broth in the range of 40.6-5200 µg/mL with sequential two-fold dilutions of (5200, 2600, 1300, 650, 325, 162.5, 81.25, 40.6 µg/mL). (Heshmati *et al.* 2015).

1) The isolates were grown on Pseudomonas agar at 37 °C for 24 h. Bacteria suspension was prepared according to (3.2.5.3).
2) The Zinc oxide nanoparticles solution, that it was used in the current study prepared and diluted in Mueller Hinton Broth (5200 µg/mL) to (stock 2).

3) 100 µL of Mueller Hinton Broth (1x) was added into all wells. A 100 µL of (stock 2) antibiotic solution moved into the wells of row 1.
4) A hundred µL was withdrawn from row 1 and add to row 2. Mix by sucking and transfer to row 3 and so on until row 8 to get 8 dilutions with a concentration of (5200, 2600, 1300, 650, 325, 162.5, 81.25, 40.6 µg/mL). A hundred µL was discard from row 8.
5) A 10 µL of bacteria suspension was added into every well in rows 1 to 8 and row 12 (positive control).
6) Added A 100 µL from stock 2 and sterile broth to the wells in row 11 and as negative controls.
7) The plate was incubated at 37 °C for 24 h.
8) A micropipette was used to mix the ZnO NPs by sucking up and down 5-8 times.

The interpretation of results had been made by hand by means of a black card and electronically with an ELISA reader on 630 nm wavelength.

4 RESULTS AND DISCUSSION

4.1 Structural, Morphological and Chemical Properties of ZnO NPs

4.1.1 Fourier transformed infra-red spectroscopy results

The results showed that there is a band with a wavelength (3306.04 cm^{-1}) when the ZnO NPs set by sol-gel technique were measured by the Fourier Transformed Infra-Red spectroscopy as shown in Figure 4.1, the results indicate the presence of hydroxyl group that belong to the water particle and the occurrence of interest band of (422.41^{-1}), these results are in agreement with the finding of (Jasim 2021, Feng *et al.* 2016).

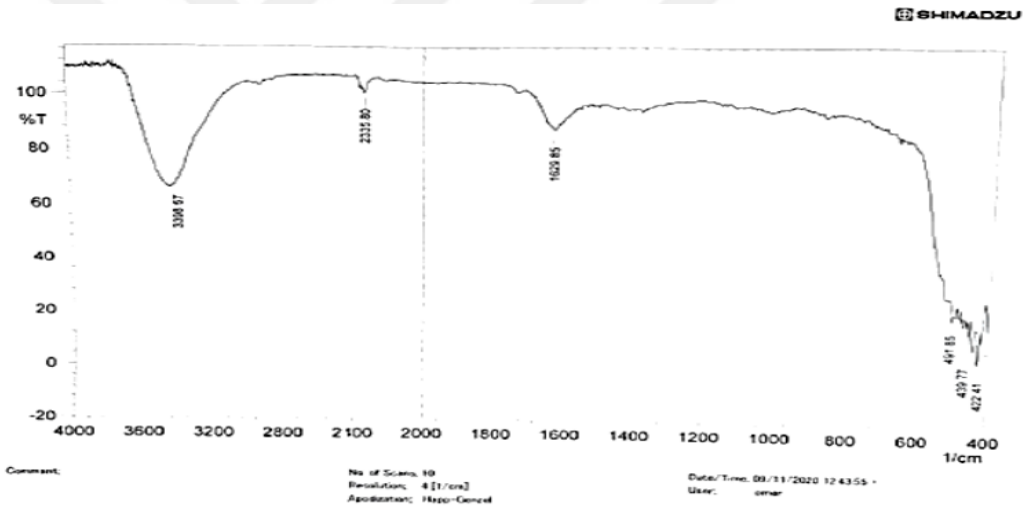


Figure 4.1 Fourier Transformed Iinfra Red for Zinc Oxide Nano Particls

4.1.2 X-ray results

The results of the X-ray diffraction patterns for the Zno NPs showed the peaks sites which are {(100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202)} and these peaks are located on the angles {(68.0466), (66.4902), (62.8531), (56.6779), (47.6492), (36.3278), (34.4759), (31.8686), (72.5937), (69.1559)} and (2θ = 77.0781) respectively as displayed in Figure 4.2. When matching the direction of these peaks and

angles with the universal card numbered (36-1451 for the Zno NPs as shown in Figure 4.3, we notice that it match with the global card significantly and these results are in agreement with (Jasim 2021, Munguti and Dejene 2021).

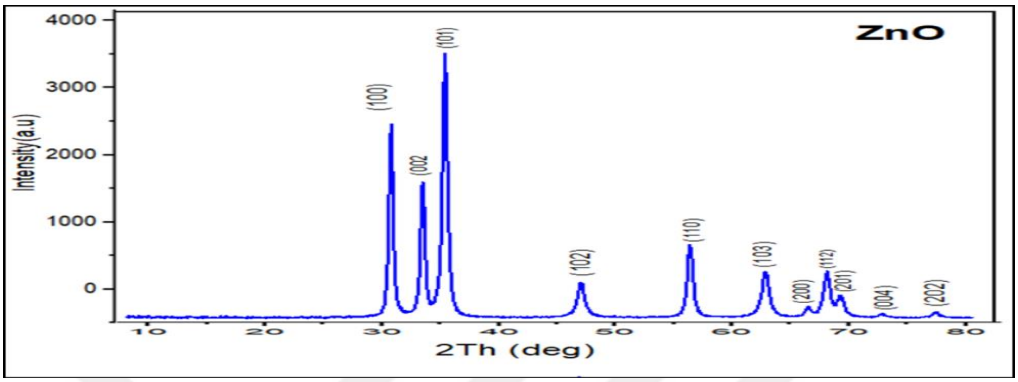


Figure 4.2 X-ray diffraction and the levels of the peaks

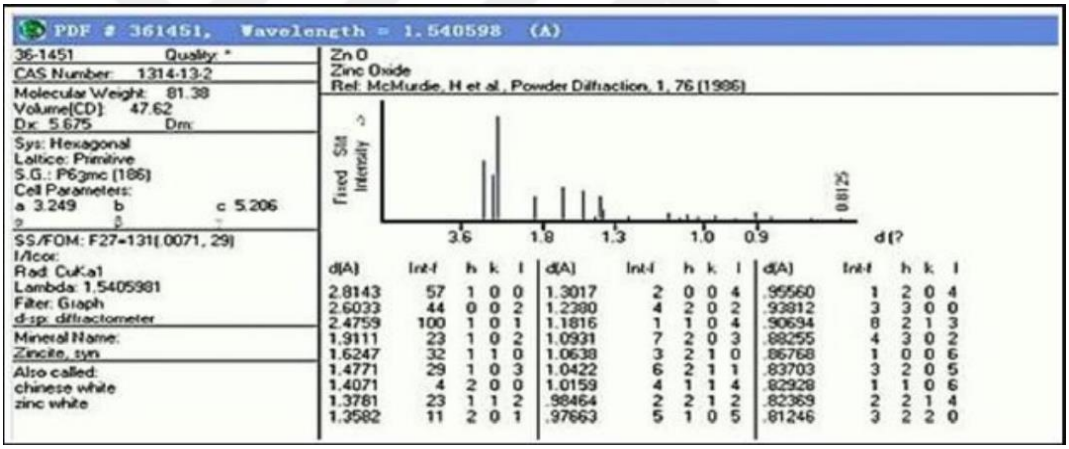


Figure 4.3 Global card for Zinc Oxide Nano Particls

The Figure 4.4 shows the results of the FE-SEM for the Zno NPs which prepared by sol-gel method, with magnifying power (50 KX), the results exhibited that utmost of the Zno NPs are compact and sphere-shaped in shape and the size of these particles range (37-62 nm), these outcomes are in covenant with the results of (Jasim 2021) and more accurate with the results of (Jurablu *et al.* 2015).

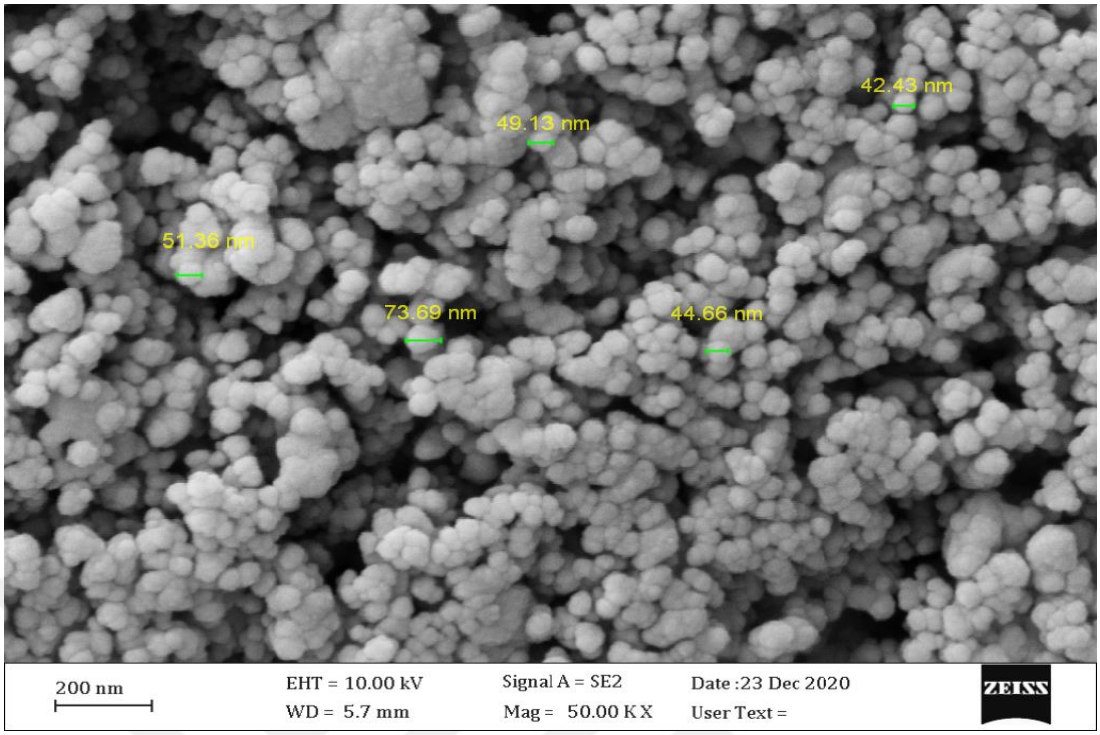


Figure 4.4 Field Emission-Scanning Eelectron Microscope for Zinc Oxide Nano Particls (42-73 nm)

4.2 Isolation of *P. aeruginosa*

In the current study, a whole number of 150 clinical cases were collected from different sources of infections included swabs of wounds and burns samples, from male and female. Patient-related information was recorded in the hospital included age, gender, and sample source as shown in Appendix 1. The swabs of clinical samples were obtained from governmental hospitals in Baqubah City/Diyala Province: Baqubah Teaching Hospital, Al-Batool Teaching Hospital and Public Health Laboratory. The isolates were collected through the study period from October, 2021 to January, 2022. The positive growth was 121 (81 %) samples while the negative growth (no growth) gave 29 (19 %) samples Figure 4.5. 25 (16.6%) *P. aeruginosa* isolates were obtained from 217 (77.5%) positive growth samples identified by morphological and biochemical test and confirmed by using VITEK® 2 Compact Automated System.

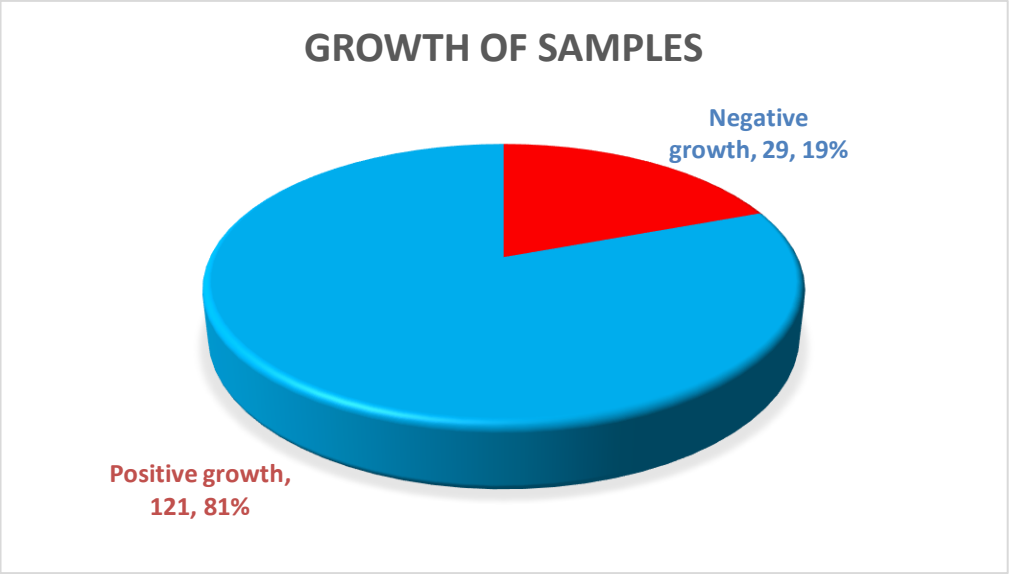


Figure 4.5 Percentage growth of *P. aeruginosa* among samples

The findings of the existing study was in covenant with the percentage of positive growth, negative growth and the isolation rate of *P. aeruginosa* of (Alhayali 2021) in Diyala province

4.2.1 Distribution of specimens and isolates according to source

The outcomes of the existing study showed that 16 (20%) of *P. aeruginosa* isolates were from burns and 9 (13%) were from wounds, this results was in agreement with Saleh (2021) who report that the highest percentage of *P. aeruginosa* isolates were from burns source (Table 4.1)

Table 4.1 Distribution of isolates according to source

Source of collection	Total number of the specimens	No. of positive *P. aeruginosa* Isolates	percentages of *P. aeruginosa* Isolates
Burns infections (Swab)	80	16	20 %
Wounds infections (Swab)	70	9	13 %
Total	150	25	17 %

Many Iraq's studies have shown that the frequency of *P. aeruginosa* in burn infections was high, as it ranged between 32-66% in provinces of Karbala, Kirkuk, Hilla and Baghdad, with the highest percentage recorded in Baghdad, Karbala, Hilla and Kirkuk (Al-Khafaji 2016, Khorsheed and Al Abdeen 2017). Also, *P. aeruginosa* isolated from wound infections recorded as highest percentage (23.5%) among the other bacterial genera isolated in Baghdad Province.

4.2.2 Morphological characteristics

All the (25) isolates were cultured on Blood agar, MacConky agar and Nutrient agar and the bacteria was then confirmed by culturing on Pseudomonas agar at 37°C for 24 hrs. The colony of the bacterium *P. aeruginosa* appeared Blood agar, a differential medium that can be distinguish bacteria according to their capability of lysing the red blood cells (RBCs), the colonies were round, convex, and surrounded by translucent halo on blood agar which indicate complete hemolysis (β-hemolysis) and this agrees with what he mentioned (Procop *et at.* 2017).

On MacConkey agar, as a selective (inhibit most Gram positive bacteria and allow to grow Gram negative bacteria) and so a differential (between lactose ferment gram negative and non-lactose ferment gram negative) in which *P. aeruginosa* non-lactose ferment as light greenish and lactose non-fermenter this agrees with what he mentioned (Al-Daraghi and Al-Badrwi 2020).

On the Nutrient agar medium a raised appearance and flat edges, smooth in shape, smell like grapes, and most of which produce Pyocyanin. To approve the diagnosis, the specimens were re-cultured on the selective Pseudomonas agar medium of this bacterium, the colonies were mucoid, smooth in shape, and have a fruity odor, and the furthermost of the bacterial isolates exhibited their capability to produce Pyocyanin (blue-green dye) and Pyoverdin dyes (fluorescent pigment) while in non-pigmented isolates were described by their unvarying circular shape and cream color. Pseudomonas agar a specific selective medium designed to select *P. aeruginosa* from other Pseudomonas species. The colonies grown on Mueller-Hinton agar show their ability to

produce dyes and especially blue-green dye (Pyocyanin). These results agree with (Alsaadi 2020, Al-Shamaa *et al.* 2016). These pigments play a part of the pathogenesis of *P. aeruginosa* as virulence factors and are also a character of pigmentation, which consider an essential issue among the diagnostic features in the genus *Pseudomonas*.

4.2.3 Biochemical tests

Biochemical tests were performed on all 25 isolates, that are included the oxidase test, catalase test, triple sugar iron (TSI) test, and IMVIC tests (indole test, methyl red test, Voges-Proskauer test, and citrate). *P. aeruginosa* was characterized by being positive to oxidase test and dark purple had appeared on the surface of colonies indicates cytochrome oxidase production and used detects the bacteria that produce the cytochrome oxidase enzyme. Bubble formation indicates a positive result for the catalase check and this check was used to detect micro-organisms that yield the enzyme catalase. Positive to citrate test by conversion of the medium to blue the result of consuming citrate. While all the isolates were negative to indol, methyl red and Voges-Proskauer. TSI test, it was establish that the entire isolates under study were not fermented for any of the 3 types of sugars (glucose, lactose, sucrose), it does not form CO2 and does not H2S production, and this was in agreement with what is revealed by (Alageedi 2021). (Table 4.2).

Table 4.2 Biochemical tests of *P. aeruginosa*

No.	Media and test	Results
1	Growth on MacConkey agar	Positive
2	Lactose fermentation	Negative
3	β-hemolysis when growth on blood agar	Positive
4	Growth on Pseudomonas agar	Positive
5	Growth at 42°C and 4°C	Positive/Negative
6	Pigment	bluish-green pigmentation
7	Gram stain	Negative
8	Oxidase	Positive
9	Catalase	Positive
10	Indole test	Negative
11	Methyl red test	Negative
12	Voges-Proskauer	Negative
13	Citrate	Positive

14	Triple sugar iron	Alk / Alk, No H_2S, No gas

4.2.4 Biochemical tests by VITEK 2

VITEK2 compact system was employed for the identification of 25 isolates using the identification card (GN ID Card) to confirm a bacterial diagnosis which gives diagnostic results for bacteria with an accuracy of 99% and contains 64 biochemical tests. All isolates were identified as *P. aeruginosa*. An example report resulting from this system for identifying these bacteria is shown in the Appendix 2. Figure 4.6 shows the diagnostic results of this device. The device shows the results of weak isolates towards a specific reaction and is denoted by the symbol (-) indicating a negative reaction and the symbol (+) indicating a positive reaction.

Test type	Result	Test type	Result	Test type	Result	Test type	Result	Test type	Result	Test type	Result
APPA	-	ADO	-	PyrA	-	IARL	-	dCEL	-	BGAL	-
H2S	-	BNAG	-	AGLTp	-	dGLU	+	GGT	+	OFF	-
BGLU	-	dMAL	-	dMAN	+	dMNE	+	BXYL	-	BAIap	+
ProA	+	LIP	+	PLE	-	TyrA	+	URE	-	dSOR	-
SAC	-	dTAG	-	dTRE	-	CIT	+	MNT	+	5KG	-
ILATK	+	AGLU	-	SUCT	+	NAGA	-	AGAL	-	PHOS	-
GLyA	-	ODC	-	LDC	-	IHISa	-	CMT	+	BGUR	-
O129R	+	GGAA	-	IMLTa	+	ELLM	-	ILATa	+		

Figure 4.6 Diagnosis by VITEK

4.2.5 Virulence factor of *P. aeruginosa*

The ability of bacterial isolates to cause infections is due to the virulence factors play that a critical role in their infections but does not participate in bacterial growth. In this

study, hemolysin, pigment production and biofilm formation are detected for the *P. aeruginosa*.

4.2.5.1 Hemolysin

The isolates under study were experienced for their capability to yield hemolysin by cultivating them on a blood agar base medium containing 5% human blood, as it was found that all isolates 25 (100%) were capable of producing beta-hemolysis around the colonies and this result agrees with (Najeeb 2020, Alageedi 2021) which was 100% and this result disagrees with (Rodulfo *et al.* 2019) which was 83.3%. Hemolysin production is the most important virulence factor for *P. aeruginosa*, as its production is associated with neurotoxicity and cytotoxicity of the cell and can destroy red blood cells to extract iron from them and it causes straight cyto-toxic effects on the renal epithelium leading to scarring. Also, hemolysins destroy various host tissues and cells including red blood cells, leucocytes, epithelial cells, and endothelial cells. It is a pore-forming toxin capable to destroy cells by lysis (Iseppi *et al.* 2020).

4.2.5.2 Pigment production

Pigment production as a virulence factor was conducted by using Pseudomonas agar and Nutrient agar. Pigments serve an imperative role in the pathogenicity of *P. aeruginosa*. Which was found 21 (84%) of isolates production of Pyocyanin on both medium this result agree with (Alageedi 2021) and only four isolates not pigment producing.

4.2.5.3 Biofilm formation

Biofilm formation is one more virulence factor for the reason that the innate resistance of the bio-film to anti-microbial agents and the choice of phenotypic variants. All isolates (25) were evaluated based on their ability to biofilms formation using the micro-titer plate technique. The micro-titer Plate technique (MTP) is a process for

studying initial bio-film formation on abiotic surfaces and it is a colorimetric performance which uses stains like the crystal violet to stain the sticky bio-films and to enumerate by using an absorbance micro-titer plate reader (Jesus and Dedeles 2020). The results of the current study presented in Table 4.3 showed that the majority of the isolates produced biofilm 23 (92%) with different yields between strongly and moderately compared to the negative control, while only two isolates (8%) represented non-biofilm producer. The absorbency values were ranged from (0.055 – 0.152) for biofilm-produced isolates and (0.037 - 0.039) for non-biofilm-produced isolates. Among biofilm-produced isolates, 36% of isolates were strongly biofilm producer this result was in agreement with (Alageedi 2021, Abdulhaq *et al.* 2020) which was 36.5%, and it is noteworthy that the rate of strong biofilm production in urine specimens was higher than other specimens and this result agrees with (Bahador *et al.* 2019). While 56% moderately biofilm producer this result agrees with (Al-Sheikhly *et al*. 2019) which was 56%. The results in the current study disagree with (Bahador *et al.* 2019) which was, 60% were powerfully bio-film produced and the rates of moderately and weak bio-film produced were 34.3% and 4.3%.

Table 4.3 Biofilm formation of *P. aeruginosa*

No. of isolate	Source of isolate	Absorbance at 360 nm	Biofilm level compared to (ODc=0.040)
P1	Wounds	0.063	Moderate
P2	Wounds	0.067	Moderate
P3	Burns	0.065	Moderate
P4	Wounds	0.069	Moderate
P5	Wounds	0.91	Strong
P6	Burns	0.071	Moderate
P7	Wounds	0.068	Moderate
P8	Burns	0.039	Negative
P9	Wounds	0.037	Negative
P10	Burns	0.079	Moderate
P11	Burns	0.058	Moderate
P12	Burns	0.055	Moderate
P13	Burns	0.064	Moderate
P14	Burns	0.152	Strong
P15	Wounds	0.066	Moderate
P16	Burns	0.121	Strong
P17	Burns	0.072	Moderate
P18	Burns	0.113	Strong
P19	Burns	0.146	Strong
P20	Wounds	0.986	Strong
P21	Burns	0.846	Strong

P22	Burns	0.146	Strong
P23	Burns	0.056	Moderate
P24	Wounds	0.069	Moderate
P25	Burns	0.934	Strong

The process of biofilm formation rest on on many influences such as the medium, the detection technique used and the incubation conditions as well as the type of surface used for that process, as the difference in the composition of the biofilm on the microtiter plate is due to the quality of the surface composition of the materials on which the biofilm is formed, for example, the membrane formed on the polystyrene surface of microtiter plates is more effective than the silicone surface of the catheter (Wojnicz *et al.* 2015). The differences in biofilm density between the isolates in this study may be due to numerous causes. Variances in the ability of strains to consist bio-films or possibly variances in the initial quantity of cells that successfully adhere to and differences in the quality and amount of quorum-sensing signaling molecules formed from every strain play important roles (Abdulammer 2018) On the other hand, (Heydari and Eftekhar 2015) reported that the dissimilarity in the capability of isolates to procedure bio-film is the reason for the correlation of production with its capability to produce different types of ß-Lactamase, which leads to the formation of a strong biofilm compared with the isolates that produced one type of enzyme. Conversely, the isolates did not produce this enzyme and were not able to form a biofilm. This in height throughput of the bio-film development may be related to the susceptibility of the microtiter plate method to quantify the insufficient amounts that formed. Which consider more accurate, easy, and sensitive in the detection of biofilm formation. When studying the early stages in biofilm formation, it is possible to adopt the microtiter plate method because this method uses stable conditions, so it can be used in the study of many factors necessary for the formation of the biofilm, such as flagellum, pili, as well as the genes that serve an imperative role in the production of exopolysaccharides (Obaid 2019).

Bio-film is representing combinations sheathed in a self-produced extra-cellular matrix that is hard to or impossible to eliminate with antibiotics. Its matrix provides a

protective border that allows it to adhere to an environmental substrate. This coating plays a role in bacteria's resistance to antibiotics. Biofilms can provide 10 to 1,000 fold more protection against antibiotic treatment. The risks of biofilm arose from the fact that it is a major driver of the persistence of chronic infection (Ciofu and Tolker-Nielsen 2019). The effect of the high percentage of the susceptibility of *P. aeruginosa* isolates in the present study on the production of biofilms demonstrates the high resistance rates for these isolates towards all antibiotics. Bacteria able to develop the resistance up to 1.000-fold more resistant to antibiotics than those in a planktonic state (Cepas and Soto 2020).

4.2.6 Antibiogram pattern

Antibiotic susceptibility test was performed for 25 isolates of *P. aeruginosa* against (Figure 4.7, Appendix 3) 12 kinds of antibiotics Norfloxacin (10 µg), Ciprofloxacin (5 µg), Imipenem (10 µg), Aztreonam (30 µg), Polymixin B (300 µg), Cefepime (30 µg), Piperacillin/Tazobactam (100/10µg), Amikacin (30 µg), Gentamicin (10 µg), Tobramycin (10 µg) related to 7 different

classes. The standard agar disk diffusion method known as the Kirby Bauer technique was used according to the "Clinical & Laboratory Standards Institute" (CLSI 2021).

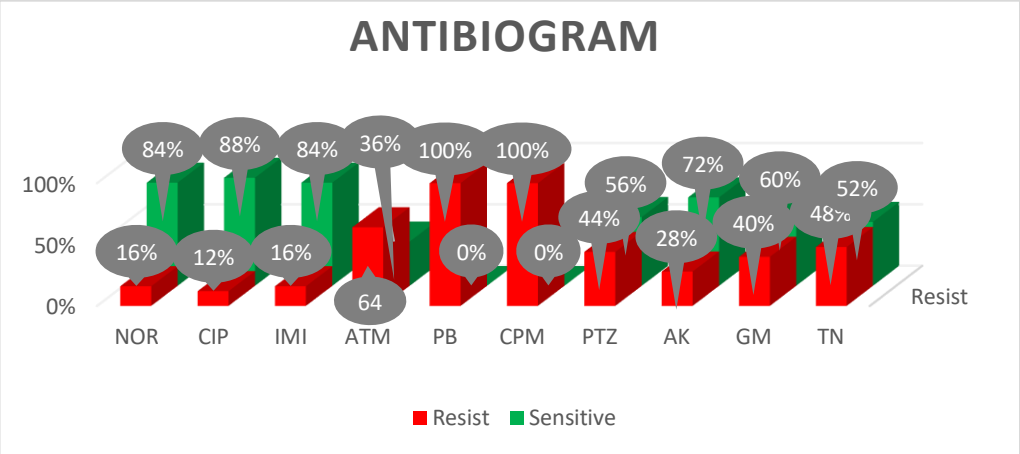


Figure 4.7 Antibiogram of *P. aeruginosa*

In the current study, five classes of cell wall synthesis inhibitors were tested and the results exhibited that the *P. aeruginosa* strains were resist to **Imipenem (IMI)** by 16%, this finding were in agreement with (Alsaadi 2020) who reported that *P. aeruginosa* isolates were resisted to IMI by 11%, and disagreement with (Al-Taee 2019) who reported that *P. aeruginosa* isolates were resisted to IMI by 63%.

Aztreonam (ATM) was resisted in 64% of *P. aeruginosa* isolates, this findings were in agreement with (Saleh 2021) who reported that *P. aeruginosa* isolates were resisted to ATM by 75%, and dis-agree with (Alageedi 2021, Alhayali 2021) who reported that *P. aeruginosa* isolates were resisted to ATM 38% and 37% respectively.

Cefepime (CPM) which was the highest percentage of resistance and was resisted in 100% of *P. aeruginosa* isolates, this findings were dis-agree with (Aziz *et al.* 2019, Ferman 2019, Mohamed *et al.* 2019, Alageedi 2021) who reported CPM resistance which was 35% and 38% 57% and 38% respectively.

Piperacillin/tazobactam (PTZ) was resisted in 44% of *P. aeruginosa* isolates, this findings were fairly close to the results of (Saleh 2021, Elhariri *et al.* 2017) who reported that *P. aeruginosa* isolates were resisted to PTZ by 66% and 76% respectively, and dis-agree with (Hoque *et al.* 2015, Sala *et al.* 2019, Alageedi 2021) who reported PTZ resistance which was 3.37%, 2%, and 3.8% respectively.

Polymixin B (PB) which was the highest percentage of resistance and acts on the outer cellular membrane of *P. aeruginosa* was resisted in 100%, this findings were in covenant with (Wickremasinghe *et al.* 2021) who informed that all *P. aeruginosa* isolates were resisted to PB.

Five classes of cell protein synthesis inhibitors were tested and the results exhibited that the *P. aeruginosa* strains were resist to **Norfloxacin (NOR)** by 16%, this findings were dis-agree with (Al-Taee 2019, Saleh 2021) who reported that NOR was resisted in *P. aeruginosa* isolates by 71% and 50% respectively.

Ciprofloxacin (CIP) which was the most effective antibiotic used and was resisted in 12% of *P. aeruginosa* isolates, this findings were dis-agree with (Qayoom *et al.* 2019, Yang *et al.* 2020, Alageedi 2021, Alhayali 2021, Saleh 2021) who reported that the resistance of *P. aeruginosa* isolates to CIP were 72%, 34%, 34%, 37%, 50% respectively.

Amikacin (AK) was resisted in 28% of *P. aeruginosa* isolates, this results was in covenant with (Macin *et al.* 2017, Sameet *et al.* 2020, Alageedi 2021) who reported that AK resistance was 29.5%, 27.2% and 30% respectively, while this result, dis-agrees with (Saleh *et al.* 2020) which was 60%.

Gentamicin (GM) was resisted in 40% of *P. aeruginosa* isolates, this results was fairly close to (Al-Obaidi and Al-Dahmoshi 2020, Hasan *et al.* 2020) who informed that the resistance of *P. aeruginosa* to GM was 50% and 30% respectively, and dis-agree with (Alsaadi 2020) who reported that *P. aeruginosa* resistance to GM was 69%.

Tobramycin was resisted in 48% of *P. aeruginosa* isolates, this results was fairly close to (Hosu *et al.* 2020, Emaneini *et al.* 2019) who reported Tobramycin resistance which was 33% and 32.9% respectively and this result disagrees with (Asghar and Ahmed 2018) which was 17%.

P. aeruginosa can produces Cephalosporinase enzymes, which are the most important types of β-lactamase, which encode chromosome-based genes as well as other types of enzymes. These enzymes have been found in *P. aeruginosa* responsible for resistance to Aztreonam, Carbapenems, first, second generation and the third of Cephalosporins and resistance to Cephalosporins are due to the destruction of the antibiotic by beta-lactamases, reduced penetration across the outer-membrane of gram-negative bacteria, and enhanced efflux (Torok *et al.* 2009). Carbapenems are a large class of beta-lactamase and are often used as a last resort treatment for Pseudomonas infection because it exhibits great attraction for penicillin-binding protein, is constant in contrast to broad-spectrum beta-lactamases (Lee and Bradley 2019, Elshamy *et al.* 2018) and easily passes through the outer-membrane (Farhan *et al.* 2019). Carbapenems

Resistance, including Imipenem 16% was also noted in the present study. This is absolutely un-expected, because of the fact that Carbapenems consider one of the furthermost that having effectivity and among the top options for handling gram-negative infections and especially multi-drug resistance infections. Multifaceted interactions mediate many of the carbapenem resistance mechanisms and may include defeat of outer-membrane purine, overexpression of efflux, and production of Carbapenemases. Carbapenem-resistant *P. aeruginosa* isolates are often related to the greater mortality ratio for the reason that the enzyme carbapenemase that mediates resistance and a greater potential for widespread resistance spread through mobile genetic elements (Juayang *et al.* 2017).

Aminoglycosides are broad-spectrum, high potency antibiotics that are traditionally used to treat serious gram-negative bacteria such as Pseudomonas infection (Holbrook and Garneau-Tsodikova 2018). Aminoglycosides work by inhibiting protein production by binding to 16S rRNA and by disrupting the integrity of the bacterial cell membrane. Aminoglycosides resistance may occur through a change in membrane permeability thus avoiding the anti-biotic molecule from entering the bacterial cell (Sameet *et al.* 2020). Tobramycin, Gentamicin and Amikacin are consider primary or second-line first-hand medications preferred in the antibiotic access group for treatment of communal or severe clinical cases (Sharland *et al.* 2018), in particular, Tobramycin is used to eliminate the early infection in cystic fibrosis (CF) while Amikacin and Gentamicin are also used in combination treatment with extra anti-biotics for improvement the total efficacy (Ren *et al.* 2019).

Amikacin resistance causes a genetic mutation that disrupts protein production. In this situation, the mistaken forms of amino acids in the poly-peptide chain fragmented to produce the incorrect form of protein, whereas the reduction in the anti-microbial activity of Amikacin was because of the alteration of enzymes and efflux pumps and improved activity as occurred in 16S rRNA methylation (Apridamayanti *et al.* 2016). The resistance of the isolates to Aminoglycoside anti-biotics was found to vary with a specific drug, the micro-organism, its machinery of resistance, the environmental area, and numerous other factors.

Fluoroquinolones resistance is likely to. be the result of a mutation (Al-Mayali and Salman 2020) because of the selective pressure induced by the consume of Fluoroquinolones, and the variance in the percentage of Ciprofloxacin resistance is usually correlated with the frequency of consume of Fluoroquinolones and the ease of use of oral doses (Mohamed *et al.* 2019).

In the current study, a lower prevalence of antibiotic resistance was observed. In dissimilarity to other studies that showed a high prevalence of antibiotic resistance see. This could occur due to the differences in *P. aeruginosa* isolates obtained and may be correlated to variances in anti-biotic use in different environments and selective pressure and this result may be due to the difference in sample size for these bacterial isolates in the current study (Mwinyikombo 2018). In general, the resistance of different types of antibiotics is attributed to many reasons, including altered cell membrane permeability, alterations in target site structures (Aziz *et al*. 2019).

4.2.7 Multi-drug resistance of *P. aeruginosa*

Multi-Drug Resistance (MDR) is defining as any isolate of bacteria that resistance against at least one antibiotic in three or more classes called Multi-Drug Resistance (Hawkey *et al.* 2018). Among 25 isolates of *P. aeruginosa* that were tested in our study, several strains were found to be MDR.

The following antipseudomonal classes of antimicrobial drugs were tested in the current study: antipseudomonal Cephalosporins (Cefepime), antipseudomonal Beta_lactamase combination inhibitors (Piperacillin/Tazobactam), antipseudomonal Monobactam (Aztreonam), antipseudomonal Carbapenems (Imipenem), antipseudomonal Lipopeptides (Polymixin B), antipseudomonal Aminoglycosides (Gentamicin, Tobramycin, Amikacin,) and Fluoroquinolones (Ciprofloxacin and Norfloxacin).

The current study results showed that 40% of isolates were MDR that agrees with (Abdallah and Gabur 2021, Alageedi 2021), who found that the percentage of MDR in

their isolates were 46% and 42% respectively, and this result disagrees with (Abd El-Baky *et al*. 2020) that found 96% of isolates were MDR. Table 4.4 shows, the most multidrug-resistant isolates.

Table 4.4 MDR, XDR and PDR of *P. aeruginosa* isolates

No. of isolate	Source	No. of resisted	Classification
P 1	Wounds	7	XDR
P 2	Wounds	7	XDR
P 5	Wounds	3	XDR
P 6	Burns	8	MDR
P 14	Burns	10	PDR
P 16	Burns	6	XDR
P 18	Burns	7	XDR
P 20	Wounds	3	MDR
P 21	Burns	8	XDR
P 22	Burns	3	MDR

Although the rate of multi-resistance in this study was relatively low, this may be a worrisome condition that reflects a threat that limits treatment options in the treatment centers studied (Kamali *et al.* 2020).

The difference between the results of our study and the other studies attributed to the variation of the antibiotics usage policy applied in each country, and primarily the increased disaster of antibiotic misuse without proper prescriptions (Mohamed *et al.* 2019).

The outbreak of emerging MDR *P. aeruginosa* strains is related to various influences, like its innate resistance to a variety of anti-biotics, its capability to acquire determinants of antimicrobial resistance, history of surgical interventions and chronic infections, mutation in the genome of *P. aeruginosa*, and environmental conditions of the specific

area, irrational use of antibiotics, and abundant use of broad-spectrum antibiotics (Mohammadzadeh *et al.* 2017). Also, the acquisition of resistance genes by horizontal gene transmission further contributes to the occurrence of phenotypes of MDR (Moustafa *et al.* 2021).

The increased frequency of multi-drug resistant strains (MDR) has been documented as a worldwide problem through the treatment of *P. aeruginosa* infection (Gonçalves *et al.* 2017).

Infections that affected by multi-drug resistant gram-negative bacteria, especially MDR strains of *P. aeruginosa*, are known to be related to higher morbidity and mortality. Patients with nosocomial infections especially those admitted to various intensive care units usually become infected with MDR strains of *P. aeruginosa* and the continued spread of such strains poses difficult issues in infection control administration (Murugan *et al.* 2018).

4.2.8 Biological activity of ZnO NPs against *P. aeruginosa*

The ZnO NPs gave a obvious anti-bacterial influence in contrast to MDR, XDR and PDR isolates of *P. aeruginosa* as displayed in Table 4.5

The ZnO NPs showed the highest diameter of inhibition zone at concentration 100 mg/mL of *P. aeruginoas* ranging from 26 mm to 52, while the ZnO NPs at a concentration of 12.5 mg/mL was lowest in diameter of inhibition zone in contrast to the similar isolates ranging from 16 mm to 44. These results were approximately close to (Mahmoud 2020)

Zinc oxide nano-particles are highly in effectiveness for inhibition of many pathogenic bacteria (Sultan *et al*. 2015). In another study (Rauf *et al*. 2017) Indicated that the synthesis of ZnO nano-particles affects *P. aeruginosa*. The anti-microbial influence may due to important features of nano-particles specifically the higher surface volume

rate which offers better interaction with the microbial surface and offers improved activity. Furthermore, the nanometer size of nanoparticles assists their entrance into the bacterial cell membrane to enable disrupts mechanisms inside the cell.

ZnO NPs produce hydrogen-peroxides which chemically interact with cell membrane (Senthilkumar and Sivakumar 2014). The anti-microbial action of nano-particles may comprise a making of reactive oxygen species (ROS) and the accumulation of nanoparticles in the bacterial cytoplasm. ROS can be the reason for cell membrane dysfunction and cell death by oxidizing lipids of cell membrane (Dutta *et al*. 2012). However, there is an absence of information of the particular machinery of nano-particle interaction with bacterial cells.

Table 4.5 Antibacterial activity of Zinc Oxide Nano Particls

P. aeruginosa	**Zno NPs 100%(mm)**	**Zno NPs 50%(mm)**	**Zno NPs 25%(mm)**	**Zno NPs 12.5%(mm)**
P1	27	24	20	17
P2	26	24	19	17
P5	26	22	20	16
P6	52	50	48	44
P14	39	32	32	30
P16	44	40	39	37
P18	43	40	38	36
P20	39	37	35	33
P21	42	39	34	32
P22	30	27	26	25

4.2.9 Determination of minimum inhibitory concentration (MIC) of Zno NPs against multi-drug resistance of *P. aeruginosa*

Over-consuming or misuse of anti-microbials that led to the improvement of multi-drug resistant bacteria. To work around this problem “the limitations of conventional synthetic anti-microbial compounds” nano-technology characterizes an unconventional approach in emerging substitute anti-microbial agents that can professionally eradicate bacterial cells and exhibit massive potential for use in both medical and veterinary applications.

The inhibitory effects of variant concentrations of ZnO NPs on *P. aeruginosa* isolates were tested and explained in Table 4.6. MIC of ZnO NPs is ranged between (2600 - 162.5 µg/mL) on *P. aeruginosa* isolates. This result was agreed with the result of (Masoumi and Shakibaie 2018, Alsaadi 2020) reported that the values of MIC for ZnO NPs were between (80- ≥2600 µg/mL) and (325 -5200 µg/mL) respectively.

In the research done by (Alanbare *et al.* 2014) the results indicated that the concentrations (500, 1000, 5000, 10000, 20000) µg/mL were mortal to *P. aeruginosa*, while (100 and 75) µg/mL were inhibitors and the concentrations (50, 25) µg/mL were not effective. While (Bohan *et al.* 2018) indicated that the outcomes of Zinc Oxide Nano-Particles in contrast to *P. aeruginosa* describe capability of it's to inhibition growth of bacteria in dissimilar rate of bacteriostatic when used in dissimilar concentration (800-2400 µg/mL).

The biological activity of ZnO NPs as an anti-bacterial comprise binding to and destructive the bacterial membrane, penetrating into the bacteria, and producing reactive oxygen species (ROS). Consequently, it is apparent that ZnO NPs by increasing the permeability of cell membrane may cause the improvement of antibiotic effectiveness. Additional description for these outcomes would be that ZnO NPs might affect with pumping activity of efflux systems. The generation of hydrogen peroxide is a core issue of the anti-bacterial activity (Bayroodi and Jalal 2016). (Figure 4.8)

Table 4.6 Minimum Inhibition Concentration of Zinc Oxide Nano Particls

No.	MIC of Zno NPs (µg/mL)	No.	MIC of Zno NPs (µg/mL)
P 1	2600	P 16	325
P 2	325	P 18	162.5
P 5	325	P 20	325
P 6	2600	P 21	325
P 14	1300	P 22	650

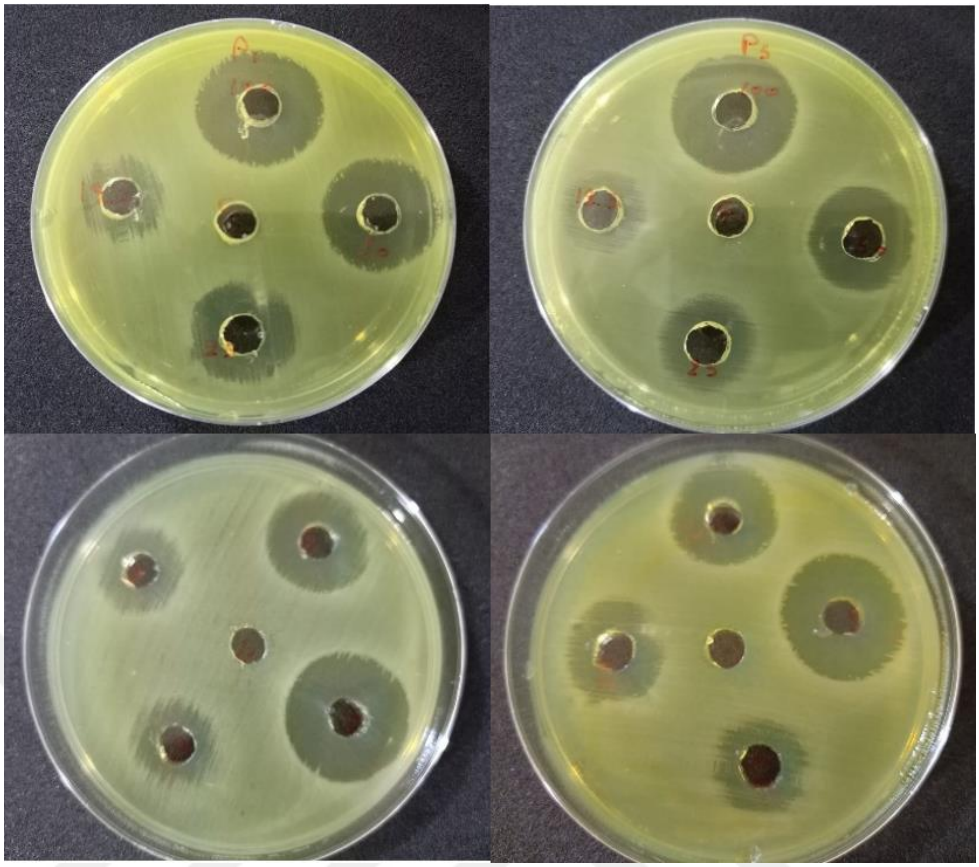


Figure 4.8 MIC and Antibacterial Activity of different concentrations from ZnO NPs by using Agar Well Diffusion method. *P. aeruginosa* on medium Pseudomonas Agar and treated by ZnONPs

The effect is clear and for all concentrations used in the study and he results of the current study were the approach with previously conducted studies on bacteria. For example, a study (Alzubaidy *et al.* 2019) whose results showed the effectiveness of the use of nanoparticles against *P. aeruginosa*, as well as (Mahdavi *et al.* 2017) showed that with the presence of ZnO NPs for both Bacillus subtilis and E. coli, the strain was significantly stabilized. They concluded that ZnO NPs showed more antimicrobial activity

.

5 CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

According to the results of the existent study, the following conclusions could be exposed:

1) *Pseudomonas aeruginosa* isolates have High resistance to Cephalosporin (Cefepime).
2) Ciprofloxacin is the most effective antibiotic against *Pseudomonas aeruginosa* in the current study.
3) The Multi-drug resistance *P. aeruginosa* become widespread in our hospitals and carrying out of infection control plans are most important worries to avoid the blowout of this dangers.
4) Zinc Oxide Nanoparticles have a strong biological activity against MDR, XDR and PDR isolates of *Pseudomonas aeruginosa*.

5.2 Recommendations

Based on the finding of the present study and after reviewing and the literature and many of previous trails the following recommendations can be suggested:

1) Periodic studies to monitor the prevalence and extent of resistance of *P. aeruginosa.*
2) Molecular study of this bacterium to understand the resistance pattern and so to overcome the spread and multi-resistant of *P. aeruginosa*
3) Prepare other types of nanoparticles and use it instead of antibiotics.

4) The importance of spreading health consciousness by warning not to use antibiotics excessively and indiscriminately and without prescription.

5) Determine the anti-bacterial inactivity of ZnO NPs on other types of pathogenic bacteria

6) The necessity of performing a laboratory analysis (culture) before prescribing any antibiotic to the patient.

REFERENCES

Abd El-Baky, R. M., Masoud, S. M., Mohamed, D. S., Waly, N. G., Shafik, E. A., Mohareb, D. A., & Hetta, H. F. 2020. Prevalence and some possible mechanisms of colistin resistance among multidrug-resistant and extensively drug-resistant *Pseudomonas aeruginosa*. Infection and Drug Resistance, 13: 323.

Abdallah, R. and Gabur, S. 2021. Molecular search of some antibiotic-borne genes of the clinical *Pseudomonas aeruginosa* plasmid. Systematic Reviews in Pharmacy,12(1):170-189.

Abdulammer, H. 2018. Detection of quinolones chromosomal resistance in *Pseudomonas aeruginosa* isolated from wounds infection. MSc. Thesis. Biology department. Baghdad University. Iraq.

Abdulhaq, N., Nawaz, Z., Zahoor, M. A., & Siddique, A. B. 2020. Association of biofilm formation with multi drug resistance in clinical isolates of *Pseudomonas aeruginosa*. EXCLI journal, 19: 201.

Agarwal, H., Kumar, S. V., & Rajeshkumar, S. 2017. A review on green synthesis of zinc oxide nanoparticles–An eco-friendly approach. Resource-Efficient Technologies, 3(4): 406-413.

Alageedi, N. M. A. 2021. The inhibition effect of antibiotics on biofilm formation by *Pseudomonas aeruginosa*. Master thesis. College of Science, University of Diyala.

Alanbare, R. A., Hussain, S. S., & Laftah, A. R. 2014. Antibacterial and Antibiofilm Activity of Zinc Oxide Nanoparticles on *Pseudomonas aeruginosa* Isolates from Diabetic Foot Infections Grade 15. Al-Mustansiriyah Journal of Pharmaceutical Sciences (AJPS), 14(2): 15-21.

Al-Dahmoshi, H. O. M. 2017. Rapid detection of microbial profile among women with vaginitis in Hilla City, Iraq. Journal of Applied Pharmaceutical Science, 7(02): 228-232.

Al-Daraghi, W. A. H., & Al-Badrwi, M. S. A. 2020. Molecular Detection for Nosocomial *Pseudomonas aeruginosa* and its Relationship with multidrug Resistance, Isolated from Hospitals Environment. Medico-Legal Update, 20(1): 661-666.

Alhayali, A. E. A. 2021. Molecular identification of Pseudomonas aeruginosa using multiple locus variable number of tandem repeat units. Master thesis. College of Science/University of Diyala.

Aljanaby, A. A. J., & Aljanaby, I. A. J. (2018). Prevalence of aerobic pathogenic bacteria isolated from patients with burn infection and their antimicrobial susceptibility patterns in Al-Najaf City, Iraq-a three-year cross-sectional study. F1000Research, 7(1157): 1157.

Al-Khafaji, M. S. A. 2016. Bacterial isolation and Physiological Aspects in Patients with Burn in AL-Hilla city. Journal of University of Babylon, 24(9).

Al-Mamari, N. M. H. 2019. Isolation and Identification of Pseudomonas aeruginosa from Some Clinical And Environmental Samples And Study It, s Activity for The Production of Pyocyanin and Protease. Journal Of Education and Science, 28(4): 93-107.

Al-Mayali, M. and Salman, E. 2020. Bacteriological and Molecular Study of Fluoroquinolones Resistance in Pseudomonas aeruginosa Isolated From Different Clinical Sources. Iraqi Journal of Science, 61(9): 2204- 2214.

Almeida, C., Azevedo, N. F., Santos, S., Keevil, C. and Vieira, M. 2013. Correction: Discriminating Multi-Species Populations in Biofilms with Peptide Nucleic Acid Fluorescence In Situ Hybridization (PNA FISH). PLoS ONE, 8 (6): 101-371.

Al-Obaidi, R. D., & Al-Dahmoshi, H. O. 2020. Biofilm and antibiotic resistance profile among Pseudomonas aeruginosa isolated from clinical samples. Eurasia J Biosci, 14(1): 1135-1139.

Al-Saadi, L. 2009. Bacteriological Study of Pseudomonas aeruginosa Isolated from Different Clinical Sources in Baaquba City and its Suburbs. M.Sc. thesis. Diyala University. Iraq.

Alsaadi, L. 2020. Molecular Detection of Multidrug-Resistant of Some Genes and the Effect of ZnONPs as Alternative to Antibiotics for Pseudomonas aeruginosa. PhD thesis. Diyala University. iraq.71-112.

Al-Saeedi, R. H. A., & Raheema, R. H. 2019. Molecular Diagnosis of some Virulence Genes in Pseudomonas aeruginosa Clinical Isolates in Wasit Province. Indian Journal of Public Health, 10(04).

Al-Shamaa, N. F., Abu-Risha, R. A., & AL-Faham, M. A. 2016. Virulence genes profile of Pseudomonas aeruginosa local isolates from burns and wounds. Iraqi journal of biotechnology, 15(3).

Al-Sheikhly, M., Musleh, L., and Al-Mathkhury, H., 2019. Assessment of peiA-carried Pseudomonas aeruginosa isolates in respect to biofilm formation. Iraqi journal of science, 60(6): 1180-1187.

Al-Shimmary, S., Jawad, N. and Wahhab, T.2017. Detection of toxA gene in clinical isolates of Pseudomonas aeruginosa using real-time PCR. Ann Trop Med Public Health, 21: SP2043-19.

Al-Taee A. K. H. 2019. Molecular study parC and gyrA genes of multi drug resistance Pseudomonas aeruginosa Isolated from Clinical Specimens. Master thesis. Unisersity of Baghdad, Institute of Genetic Engineering and Biotechnology.

Alzubaidy, M. W. M., Almohaidi, A. M. S., Sultan, A. A., & AL-Shimmary, S. M. 2019. Virulence gene of Pseudomonas aeruginosa with nanoparticle. In AIP Conference Proceedings (Vol. 2123, No. 1, p. 020039). AIP Publishing LLC.

Amoon, R. H., Abdallha, A. H., Sharif, A. O., Moglad, E. H., Altyb, H. N., Elzaki, S. G., & Salih, M. A. 2018. Molecular characterization of Pseudomonas aeruginosa isolates from Sudanese patients: A cross-sectional study. F1000Research, 7(1135): 1135.

Anbuvannan, M., Ramesh, M., Viruthagiri, G., Shanmugam, N., & Kannadasan, N. 2015. Synthesis, characterization and photocatalytic activity of ZnO nanoparticles prepared by biological method. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 143: 304-308.

Apridamayanti, P., Meilinasary, K. A., & Sari, R. 2016. Antibiotic Sensitivity in Pseudomonas aeruginosa of DiabeticPatient's Foot Ulcer. Pharmaceutical Sciences and Research, 3(2): 3.

Asghar, A. and Ahmed, O. 2018. Prevalence of aminoglycoside resistance genes in Pseudomonas aeruginosa isolated from a tertiary care hospital in Makkah, KSA. Clinicalpractice,15(2):541-547.

Aykac, K., Ozsurekci, Y., & Tanir Basaranoglu, S. 2017. Future directions and molecular basis of ventilator associated pneumonia. Canadian respiratory journal, 2017.

Aziz, M., Garduno, R., Mirani, Z. A., Baqai, R., Sheikh, A. S., Nazir, H., & Kazmi, S. U. 2019. Determination of antimicrobial effect of protamine by transmission electron microscopy and SDS PAGE on Pseudomonas aeruginosa isolates from diabetic foot infection. Iranian Journal of Basic Medical Sciences, 22(7): 827.

Baek, Y. W. and An, Y. J. 2011. Microbial toxicity of metal oxide nanoparticles (CuO, NiO, ZnO, and Sb2O3 to Escherichia coli, Bacillus subtilis, and Streptococcus aureus. Science of the Total Environment 409(8):1603-8.

Bahador, N., Shoja, S., Faridi, F., Dozandeh-Mobarrez, B., Qeshmi, F. I., Javadpour, S., & Mokhtary, S. 2019. Molecular detection of virulence factors and biofilm formation in Pseudomonas aeruginosa obtained from different clinical specimens in Bandar Abbas. Iranian Journal of Microbiology, 11(1): 25.

Baron, E. J., Finegold, S. M. and Peterson, I. L. R. 2007. Bailey and Scotts Diagnostic Microbiology. 9th. ed. Mosby Company. Missouri.

Bayroodi, E., & Jalal, R. 2016. Modulation of antibiotic resistance in Pseudomonas aeruginosa by ZnO nanoparticles. Iranian journal of microbiology, 8(2): 85.

Blair, J., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. 2015. Molecular mechanisms of antibiotic resistance. Nature reviews microbiology, 13(1): 42-51.

Bohan, A. J. 2018. Antibacterial Activity of Zinc Oxide Nano Particles against Bacteria Isolated from Infants with Urinary Tract Infection. Al-Mustansiriyah Journal of Science, 29(2): 34-42.

Brauner, A., Shoresh, N., Fridman, O., & Balaban, N. Q. 2017. An experimental framework for quantifying bacterial tolerance. Biophysical journal, 112(12): 2664-2671.

Brooks, G. F., Carrol, K. C., Butel, J. S., and Mores, S. A. 2016. Pseudomonas In: Medical Microbiology. 27th Ed. The McGraw-Hill, Inc: 245-247.

Cepas, V., & Soto, S. M. 2020. Relationship between virulence and resistance among gram-negative bacteria. Antibiotics, 9(10): 719.

Chaudhary, R., Thapa, S. K., Rana, J. C., & Shah, P. K. 2017. Surgical site infections and antimicrobial resistance pattern. Journal of Nepal Health Research Council, 15(2): 120-123.

Ciofu, O., & Tolker-Nielsen, T. 2019. Tolerance and resistance of Pseudomonas aeruginosa biofilms to antimicrobial agents—how P. aeruginosa can escape antibiotics. Frontiers in microbiology, 913.

Clinical and Laboratory Standards Institute 2021. Performance Standards for Antimicrobial Susceptibility Testing. Approved standard-30th edition. CLSI document M100.

Cohen, J., Powderly, W. G., & Opal, S. M. 2016. Infectious Diseases E-Book. Elsevier Health Sciences.

Cunha, M. T., Murça, M. A., Nigro, S., Klautau, G. B., & Salles, M. J. C. 2018. In vitro antibacterial activity of bioactive glass S53P4 on multiresistant pathogens causing osteomyelitis and prosthetic joint infection. BMC infectious diseases, 18(1): 1-6.

Dantas, G., & Sommer, M. O. 2014. How to fight back against antibiotic resistance. Am Sci, 102(1): 42-51.

De Azeredo, H. M. 2013. Antimicrobial nanostructures in food packaging. Trends in food science & technology, 30(1): 56-69.

Dutta, R. K., Nenavathu, B. P., Gangishetty, M. K., & Reddy, A. V. R. 2012. Studies on antibacterial activity of ZnO nanoparticles by ROS induced lipid peroxidation. Colloids and Surfaces B: Biointerfaces, 94: 143-150.

El Zowalaty, M. E., Al Thani, A. A., Webster, T. J., El Zowalaty, A. E., Schweizer, H. P., Nasrallah, G. K., & Ashour, H. M. 2015. Pseudomonas aeruginosa: arsenal of resistance mechanisms, decades of changing resistance profiles, and future antimicrobial therapies. Future Microbiology, 10(10): 1683-1706.

Elhariri, M., Hamza, D., Elhelw, R., & Dorgham, S. M. 2017. Extended-spectrum beta-lactamase-producing Pseudomonas aeruginosa in camel in Egypt: potential human hazard. Annals of clinical microbiology and antimicrobials, 16(1): 1-6.

Elkady, M. F., Shokry Hassan, H., Hafez, E. E., & Fouad, A. 2015. Construction of zinc oxide into different morphological structures to be utilized as antimicrobial agent against multidrug resistant bacteria. Bioinorganic Chemistry and Applications, 2015.

Elshamy, A. A., Aboshanab, K. M., Yassien, M. A., & Hassouna, N. A. 2018. Prevalence of carbapenem resistance among multidrug-resistant Gram-negative

uropathogens. Archives of Pharmaceutical Sciences Ain Shams University, 2(2): 70-77.

Emaneini, M., Kalantar- Neyestanaki, D., Jabalameli,L., Hashemi, M., Beigverdi, R. and Jabalameli, F. 2019. Molecular analysis and antimicrobial resistance pattern of distinct strains of Pseudomonas aeruginosa isolated from cystic fibrosis patients in Iran. Iran Journal of microbiology,11(2):98-107

Esmaeili, D., Daymad, S. F., Neshani, A., Rashki, S., Marzhoseyni, Z., & Khaledi, A. 2019. Alerting prevalence of MBLs producing Pseudomonas aeruginosa isolates. Gene Reports, 16: 100460.

Etebu, E., & Arikekpar, I. 2016. Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives. Int. J. Appl. Microbiol. Biotechnol. Res, 4(2016): 90-101.

Farahi, R. M., Ali, A. A., & Gharavi, S. 2018. Characterization of gyrA and parC mutations in ciprofloxacin-resistant Pseudomonas aeruginosa isolates from Tehran hospitals in Iran. Iranian journal of microbiology, 10(4): 242.

Farhan, S. M., Ibrahim, R. A., Mahran, K. M., Hetta, H. F., & Abd El-Baky, R. M. 2019. Antimicrobial resistance pattern and molecular genetic distribution of metallo-β-lactamases producing Pseudomonas aeruginosa isolated from hospitals in Minia, Egypt. Infection and Drug Resistance, 12: 2125.

Feng, X., Yan, Y., Wan, B., Li, W., Jaisi, D. P., Zheng, L., and Liu, F. 2016. Enhanced dissolution and transformation of ZnO nanoparticles: the role of inositol hexakisphosphate. Environmental science & technology, 50(11): 5651-5660.

Ferman, F. 2019.Isolation and Identification Bacteriophages of Pseudomonas aeruginosa caused Wound and Burn Infections and Study of its Antimicrobia lEffective. Msc thesis. Diyala University iraq. 42-43.

Forbes BA, Sahm DF, and Weissfeld AS (2007). Bailey and Scott′s Diagnostic Microbiology. 12th ed. Mosby, USA.

Fugelsang, K. C., & Edwards, C. G. 2000. Wine microbiology: practical applications and procedures. Boston, MA: Springer US.

Gerard, T., Funke, B. and Case, C.2016. Microbiology: An Introduction.12th ed. Pearson Education. 54.

Ghanbarzadeh, B., Oleyaei, S. A., & Almasi, H. 2015. Nanostructured materials utilized in biopolymer-based plastics for food packaging applications. Critical reviews in food science and nutrition, 55(12): 1699-1723.

Gleiter, H. 2000. Nanostructured materials: basic concepts and microstructure. Acta materialia, 48(1): 1-29.

Golle, A., Janezic, S., & Rupnik, M. 2017. Low overlap between carbapenem resistant Pseudomonas aeruginosa genotypes isolated from hospitalized patients and wastewater treatment plants. PloS one, 12(10): e0186736.

Gomila, A., Carratalà, J., Badia, J. M., Camprubí, D., Piriz, M., Shaw, E., & Pujol, M. 2018. Preoperative oral antibiotic prophylaxis reduces Pseudomonas aeruginosa surgical site infections after elective colorectal surgery: a multicenter prospective cohort study. BMC Infectious Diseases, 18(1): 1-9.

Gonçalves, I. R., Dantas, R. C. C., Ferreira, M. L., Batistão, D. W. D. F., Gontijo-Filho, P. P., & Ribas, R. M. 2017. Carbapenem-resistant Pseudomonas aeruginosa: association with virulence genes and biofilm formation. brazilian journal of microbiology, 48: 211-217.

Hasan, S. A., Najati, A. M., & Abass, K. S. 2020. Prevalence and antibiotic resistance of "pseudomonas aeruginosa" isolated from clinical samples in Kirkuk City, Iraq. Eurasia J Biosci, 14(1): 1821-5.

Hassuna, N. A., Darwish, M. K., Sayed, M., & Ibrahem, R. A. 2020. Molecular epidemiology and mechanisms of high-level resistance to meropenem and imipenem in Pseudomonas aeruginosa. Infection and Drug Resistance, 13: 285.

Hawkey, P. M., Warren, R. E., Livermore, D. M., McNulty, C. A., Enoch, D. A., Otter, J. A., & Wilson, A. P. R. 2018. Treatment of infections caused by multidrug-resistant gram-negative bacteria: Report of the British society for antimicrobial chemotherapy/healthcare infection society/british infection association joint working party. Journal of Antimicrobial Chemotherapy, 73(suppl_3), iii2-iii78.

Hemraj, V.; Diksha, S.; and Avneet, G. 2013. A review on commonly used biochemical test for bacteria. Innovare Journal of Life Science, 1(1): 1–7.

Hernández Durán, M. ,LópezJácome, L., Colín Castro C., Cerón González G., Ort gaPeña, S., and Vanegas Rodríguez, E. (2017). Comparison of the Micro Scan alk Away and VITEK2 Compact systems for the identification and susceptibility of

cinical Gram positive and Gram negative bacteria. *Investigaction En Discapacidad*,6(3):105-114.

Heshmati, J., Namazi, N., Memarzadeh, M. R., Taghizadeh, M., and Kolahdooz, F. 2015. Nigella sativa oil affects glucose metabolism and lipid concentrations in patients with type 2 diabetes: A randomized, double-blind, placebo-controlled trial. Food Research International, 70: 87-93.

Heydari, S., & Eftekhar, F. 2015. Biofilm formation and β-lactamase production in burn isolates of Pseudomonas aeruginosa. Jundishapur journal of microbiology, 8(3).

Hilliam, Y., Kaye, S. and Winstanley C. 2020. Pseudomonas aeruginosa and microbial keratitis. Journal Medical Microbiology,69(1): 3-13.

Holbrook, S. Y., & Garneau-Tsodikova, S. 2018. Evaluation of aminoglycoside and carbapenem resistance in a collection of drug-resistant Pseudomonas aeruginosa clinical isolates. Microbial Drug Resistance, 24(7): 1020-1030.

Hoque, M., Ahmed, M., Khisa, S., Uddin, M., and Jesmine, R., 2015. Antibiotic resistance Pattern In Pseudomonas aeruginosa Isolated from Different Clinical Specimens. JAFMC Bangladesh 11(1): 45-49.

Hosu, M. C., Vasaikar, S., Okuthe, G. E., & Apalata, T. 2020. Antibiotic resistant gene of Pseudomonas aeruginosa from non-clinical environment: public health implications in Mthatha, Eastern Cape Province, South Africa.

Iseppi, R., Sabia, C., Bondi, M., Mariani, M., & Messi, P. 2020. Virulence factors, drug resistance and biofilm formation in Pseudomonas species isolated from healthcare water systems. Current Microbiology, 77(8): 1737-1745.

Jabuk, S., Jabuk, N., AL-Harmoosh, R. and Hussien, R.2017.Isolation and Identification of antibiotic-producing bacteria from local soil in Babylon province. Euphrates Journal of Agriculture Science Second Veterinary Conference, 304-3010.

Jain, N., Bhargava, A., & Panwar, J. 2014. Enhanced photocatalytic degradation of methylene blue using biologically synthesized “protein-capped” ZnO nanoparticles. Chemical Engineering Journal, 243: 549-555.

Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M., Nawaz, M. A., & Kamil, M. A. 2018. Bacterial biofilm and associated infections. Journal of the chinese medical association, 81(1): 7-11.

James G. Cappuccino, E. and C. W. 2020 Provide New Learning Pathways to Understand the Why Behind the Science.

Jasim, B. M. A. 2021. Effect Of Zinc Oxide Nanoparticles On Skin Cancer Cell Lines. Master thesis, College Of Education Pure Science Diyala University

Jayaseelan, S., Ramaswamy, D., & Dharmaraj, S. 2014. Pyocyanin: production, applications, challenges and new insights. World Journal of Microbiology and Biotechnology, 30(4): 1159-1168.

Jesus, R. and Dedeles, G. 2020. Data on quantitation of Bacillus cereus sensulato biofilms by microtiter plate biofilm formation assay. Data in brief,28:104951.

Jones, N., Ray, B., Ranjit, K. T., & Manna, A. C. 2008. Antibacterial activity of ZnO nanoparticle suspensions on a broad spectrum of microorganisms. FEMS microbiology letters, 279(1): 71-76.

Juayang, A. C., Lim, J. P. T., Bonifacio, A. F. V., Lambot, A. V. L., Millan, S. M., Sevilla, V. Z. J. N., & Gallega, C. T. 2017. Five-year antimicrobial susceptibility of Pseudomonas aeruginosa from a local tertiary hospital in Bacolod City, Philippines. Tropical medicine and infectious disease, 2(3): 28.

Jurablu, S., Farahmandjou, M., & Firoozabadi, T. P. 2015. Sol-gel of zinc oxide synthesis (ZnO) nanoparticles: study of structural and optical properties. Journal of Sciences, Islamic Republic of Iran, 26(3): 281-285.

Kamal, M. A., Aldin, C. I., & Husein, A. S. 2018. Prevalence study of Pseudomonas aeruginosa in Teaching Tikrit Hospital from different sources. Tikrit Journal of Pure Science, 20(4): 55-59.

Kamali, E., Jamali, A., Ardebili, A., Ezadi, F., & Mohebbi, A. 2020. Evaluation of antimicrobial resistance, biofilm forming potential, and the presence of biofilm-related genes among clinical isolates of Pseudomonas aeruginosa. BMC research notes, 13(1): 1-6.

Kasprowicz, A., Białecka, A., & Białecka, J. 2018. Diagnostics: Routine Identification on Standard and Chromogenic Media, and Advanced Automated Methods. In Pet-To-Man Travelling Staphylococci (pp. 185-198). Academic Press.

Kaur, M., Sharma, S., & Bedi, P. M. 2015. Silica supported Brönsted acids as catalyst in organic transformations: A comprehensive review. Chinese Journal of Catalysis, 36(4): 520-549.

Khodair, Z. T., Khadom, A. A., & Jasim, H. A. 2019. Corrosion protection of mild steel in different aqueous media via epoxy/nanomaterial coating: preparation, characterization and mathematical views. Journal of Materials Research and Technology, 8(1): 424-435.

Khorsheed, M., & Al Abdeen, S. Z. 2017. The frequency of Pseudomonas aeruginosa bacteria with some pathogenic bacteria in burns injuries and study their resistance to antibiotics. Kirkuk University Journal-Scientific Studies, 12(1): 123-140.

Klockgether, J., & Tümmler, B. 2017. Recent advances in understanding Pseudomonas aeruginosa as a pathogen. F1000Research, 6.

Kordes, A., Preusse, M., Willger, S. D., Braubach, P., Jonigk, D., Haverich, A., & Häussler, S. 2019. Genetically diverse Pseudomonas aeruginosa populations display similar transcriptomic profiles in a cystic fibrosis explanted lung. Nature communications, 10(1): 1-10.

Lee, N., Lim, C. H., Kim, T., Son, E. K., Chung, G. S., Rho, C. J., & World Health Organization. 2017. Systematic evidence review for developing WHO guidelines on protecting workers from potential risks of manufactured nanomaterials: which hazard category should specific nanomaterials or groups of nanomaterials be assigned to and how?

Lee, Y., & Bradley, N. 2019. Overview and insights into carbapenem allergy. Pharmacy, 7(3): 110.

Lister, P. D., Wolter, D. J., & Hanson, N. D. 2009. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clinical microbiology reviews, 22(4): 582-610.

Luis, M., Pezzlo, M. T., Bittencourt, C. E., & Peterson, E. M. 2020. Color atlas of medical bacteriology. John Wiley & Sons.

Ma, H., Williams, P. L., & Diamond, S. A. 2013. Ecotoxicity of manufactured ZnO nanoparticles–a review. Environmental Pollution, 172: 76-85.

Macin, S., Akarca, M., Sener, B., & Akyon, Y. 2017. Comparison of virulence factors and antibiotic resistance of Pseudomonas aeruginosa strains isolated from patients with and without cystic fibrosis.

Madhumitha, G., Elango, G., and Roopan, S. M. 2016. Biotechnological aspects of ZnO nanoparticles: overview on synthesis and its applications. Applied Microbiology and Biotechnology, 100(2): 571–581

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., & Monnet, D. L. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection, 18(3): 268-281.

Mahdavi, M., Zahraei Salehi, T., Amini, K., & Mobasseri, P. 2017. Frequency of exoY, exoS, exoT and exoU genes among Pseudomonas aeruginosa Isolated from patients in Tehran hospitals by Multiplex PCR. Iranian Journal of Medical Microbiology, 11(1): 9-17.

Mahmoud, A. H. 2020. Biosysnthesis and characterization of some nanoparticles by using plants extracts and study their antimicrobial property against pathogenic bacteria isolated from wounds and burns. Master thesis.

Majeed, H. T., & Aljanaby, A. A. J. 2019. Antibiotic susceptibility patterns and prevalence of some extended spectrum beta-lactamases genes in gram-negative bacteria isolated from patients infected with urinary tract infections in Al-Najaf City, Iraq. Avicenna journal of medical biotechnology, 11(2): 192.

Masoumi, S., & Shakibaie, M. R. 2018. Evaluation synergistic effect of TiO2, ZnO nanoparticles and amphiphilic peptides (Mastoparan-B, indolicidin) against drug-resistant Pseudomonas aeruginosa, Klebsiella pneumoniae and acinetobacter baumannii. Archives of Pediatric Infectious Diseases, 6(3).

McCutcheon, R., Beck, K., Jauhar, S., and Howes, O. D. 2018. Defining the locus of dopaminergic dysfunction in schizophrenia: a meta-analysis and test of the mesolimbic hypothesis. Schizophrenia bulletin, 44(6): 1301-1311.

Mirzaei, H., & Darroudi, M. 2017. Zinc oxide nanoparticles: Biological synthesis and biomedical applications. Ceramics International, 43(1): 907-914.

Mohamed,F., Askoura, M. and Shaker,G .2019. Antibiotic susceptibility of Pseudomonas aeruginosa isolated from different clinical sources. Zagazig Journal Pharmaceutical, 28(2):10-17.

Mohammadzadeh, A., Mardaneh, J., Ahmadi, R., & Adabi, J. 2017. Evaluation of the virulence features and antibiotic resistance patterns of pathogenic Pseudomonas aeruginosa strains isolated from hospitalized patients in Gonabad, Iran. Archives of Pediatric Infectious Diseases, 5(3).

Moore, E. R., Tindall, B. J., Martins Dos Santos, V. A., Pieper, D. H., Ramos, J. L., & Palleroni, N. J. 2006. Nonmedical: pseudomonas. The prokaryotes, 6: 646-703.

Moore, N. M., & Flaws, M. L. 2011. Antimicrobial resistance mechanisms in Pseudomonas aeruginosa. Clinical Laboratory Science, 24(1): 47.

Moreno E., Prats G., Sabaté M., Pérez T., Johnson J. R., and Andreu A. 2006. Quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole resistancein relation to virulence determinants and phylogenetic background among uropathogenic Escherichia coli. Journal of Antimicrobial Chemotherapy. 57(2):204–211.

Moustafa, D. A., Wu, A. W., Zamora, D., Daly, S. M., Sturge, C. R., Pybus, C., & Greenberg, D. E. 2021. Peptide-conjugated phosphorodiamidate morpholino oligomers retain activity against multidrug-resistant Pseudomonas aeruginosa in vitro and in vivo. MBio, 12(1): e02411-20.

Mukherjee, S., Moustafa, D. A., Stergioula, V., Smith, C. D., Goldberg, J. B., & Bassler, B. L. 2018. The PqsE and RhlR proteins are an autoinducer synthase–receptor pair that control virulence and biofilm development in Pseudomonas aeruginosa. Proceedings of the National Academy of Sciences, 115(40): E9411-E9418.

Mun, I. R. A. 2009. Outbreak of Multidrug-Resistant Pseudomonas aeruginosa Isolates from Burn Infections and Detection of Some Virulence Factors. Al-Mustansiriyah Journal of Science, 20(4).

Munguti, L., & Dejene, F. 2021. Effects of Zn: Ti molar ratios on the morphological, optical and photocatalytic properties of ZnO-TiO2 nanocomposites for application in dye removal. Materials Science in Semiconductor Processing, 128: 105786.

Murray, P., Rosenthal, K. and Faller, P. 2021. Medical Microbiology, 9ed, USA: Elsevier, 27-29.

Murugan, N., Malathi, J., Therese, K. L., & Madhavan, H. N. 2018. Application of six multiplex PCR's among 200 clinical isolates of Pseudomonas aeruginosa for the

detection of 20 drug resistance encoding genes. The Kaohsiung Journal of Medical Sciences, 34(2): 79-88.

Mwinyikombo, S. I. 2018. Isolation, Antibiotic Susceptibility and Molecular Characterisation of resistance genes in Pseudomonas isolates from selected Hospitals in Mombasa County.

Nader, M. I., Kareem, A. A., Rasheed, M. N., & Issa, M. A. 2017. Biofilm Formation and Detection of pslÁ Gene in Multidrug Resistant Pseudomonas aeruginosa Isolated from Thi-Qar, Iraq. Iraqi journal of biotechnology, 16(4).

Najeeb, A. 2020. Genotyping Diversity of Pseudomonas aeruginosa Isolated from Clinical specimens in Baquba. MSCthesis, University of Diyala, Iraq, 58-97.

Najnin, A., Farzana, Y., Rashid ,M., Deepthi ,S., Srikumar ,C.,Rashid.S., Nazmul, M. and Fazlul , M. 2018. Detection of virulence factors and β lactamase encoding genes among the clinical isolates of Pseudomonas nanocomposites for application in dye removal. Materials Science in Semiconductor Processing, 128: 105786.

Nielsen, S. L. 2015. The incidence and prognosis of patients with bacteremia. Infection, 70(2): 111-126.

Obaid, K. 2019. Effect of Lactobacillus acidophilus Supernatant on the Biofilm Formation of Some Pathogenic Bacteria. Journal of University of Babylon for Pure and Applied Sciences,27(4):72-90.

Obedat, M., Shatnawi, M., Al-Alawi, M., Al-Zubi, E., Al-Dmoor, H., Al-Qudah, M., El-Qudah, J., and Qrti, I., 2012. Antimicrobial activity of crude extracts of some plant leaves. Res. J. of Microbiology, 7:59-67.

Orlandi, V. T., Bolognese, F., Chiodaroli, L., Tolker-Nielsen, T., & Barbieri, P. 2015. Pigments influence the tolerance of Pseudomonas aeruginosa PAO1 to photodynamically induced oxidative stress. Microbiology, 161(12): 2298-2309.

Osman, D. A. M., & Mustafa, M. A. 2015. Synthesis and characterization of zinc oxide nanoparticles using zinc acetate dihydrate and sodium hydroxide. Journal of Nanoscience and Nanoengineering, 1(4): 248-251.

O'Toole, G. A. 2018. Cystic fibrosis airway microbiome: overturning the old, opening the way for the new. Journal of bacteriology, 200(4): e00561-17.

Pachori, P., Gothalwal, R. and Gandhi, P.2019. Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit a critical review. ScienceDirect, 6(2) :109-119.

Palavutitotai, N., Jitmuang, A., Tongsai, S., Kiratisin, P. and Angkasekwinai, N. 2018. Epidemiology and risk factors of extensively drug-resistant Pseudomonas aeruginosa infections. PLoS One, 13 (2): e019343.

Pang, Z., Raudonis, R., Glick, B. R., Lin, T. J., & Cheng, Z. 2019. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. Biotechnology advances, 37(1): 177-192.

Procop, G., Church, D., Hall, G., Janda, W., Koneman, E., Schreckenberger, P. and Woods, G. 2017. Color atlas and textbook of diagnostic microbiology. 7th.ed. Philadelphia: Wolters Kluwer Health.

Procop, G., Church, D., Hall, G., Janda, W., Koneman, E., Schrecken, B. and Woods, G. 2016. Koneman's color atlas and textbook of diagnostic microbiology. 7th ed. Lippincott Williams and Willkins. Philadelphia. Baltimore. New York.Pseudomonas aeruginosa. Journal of microbiological methods, 127: 182-187.

Purro, M., Qiao, J., Liu, Z., Ashcraft, M., & Xiong, M. P. 2018. Desferrioxamine: gallium-pluronic micelles increase outer membrane permeability and potentiate antibiotic activity against Pseudomonas aeruginosa. Chemical Communications, 54(99): 13929-13932.

Qayoom, S., Rashid, A., Kohli, A., Masoodi, T., & Amin, M. 2019. Prevalence and antibiotic sensitivity pattern of Pseudomonas aeruginosa isolates from respiratory samples, pus samples and body fluids in a tertiary care hospital, Kashmir. Indian J. Microbiol. Res, 6(4): 345-349.

Rauf, M. A., Owais, M., Rajpoot, R., Ahmad, F., Khan, N., & Zubair, S. 2017. Biomimetically synthesized ZnO nanoparticles attain potent antibacterial activity against less susceptible S. aureus skin infection in experimental animals. RSC advances, 7(58): 36361-36373.

Reda, F. M., El-Zawahry, Y. A., & Omar, A. R. 2017. Synergistic effect of combined antibiotic and methanol extract of Eucalyptus camaldulensis leaf against

Staphylococcus aureus and Pseudomonas aeruginosa. International Journal of Applied Sciences and Biotechnology, 5(4): 486-497.

Ren, H., Liu, Y., Zhou, J., Long, Y., Liu, C., Xia, B., & Wu, W. 2019. Combination of azithromycin and gentamicin for efficient treatment of Pseudomonas aeruginosa infections. The Journal of Infectious Diseases, 220(10): 1667-1678.

Riedel, S., Morse, S. A., Mietzner, T. A., & Miller, S. 2022. Microbiologia Médica de Jawetz, Melnick & Adelberg-28. McGraw Hill Brasil.

Rocha, A. J., Barsottini, M. R. D. O., Rocha, R. R., Laurindo, M. V., Moraes, F. L. L. D., & Rocha, S. L. D. 2019. Pseudomonas aeruginosa: virulence factors and antibiotic resistance genes. Brazilian Archives of Biology and Technology, 62.

Rodulfo, H., Arcia, A., Hernández, A., Michelli, E., Martinez, D. D. V., Guzman, M., ... & Donato, M. D. 2019. Virulence factors and integrons are associated with MDR and XDR phenotypes in nosocomial strains of Pseudomonas aeruginosa in a Venezuelan university hospital. Revista do Instituto de Medicina Tropical de São Paulo, 61.

Sabir, S., Arshad, M., & Chaudhari, S. K. 2014. Zinc oxide nanoparticles for revolutionizing agriculture: synthesis and applications. The Scientific World Journal, 2014.

Sahayaraj, K., and Rajesh, S. 2011. Bionanoparticles: synthesis and antimicrobial applications. Science against microbial pathogens: communicating current research and technological advances, 23: 228-244.

Sala, A., Di Ianni, F., Pelizzone, I., Bertocchi, M., Santospirito, D., Rogato, F., & Cabassi, C. S. 2019. The prevalence of Pseudomonas aeruginosa and multidrug resistant Pseudomonas aeruginosa in healthy captive ophidian. PeerJ, 7: e6706.

Saleh, R. M. 2021. Detection of exoA and oprD genes expression in clinical isolates of Pseudomonas aeruginosa. Master thesis. College of Medicine-University of Diyala

Saleh, T., Hashim, S. ,Al-Obaidi1, R.and AL-Rubaii, B.2020. A biological study of chitinase produced by clinical isolates of Pseudomonas aeruginosa and detection of ChiA responsible gene. International Journal Research Pharmaceutical Sciences,11(2):1318-1330.

Sameet, M. C., Awadh, H. A., & Suleiman, A. A. 2020. Effect of Isolation Source on Virulence Gene Expression, Phenotypic and Antibiotic Resistance Patterns of Clinical Isolate of Pseudomonas aeruginosa. Annals of Tropical Medicine and Public Health, 23: 184-189.

Schroeder, M., Brooks, B. D., & Brooks, A. E. 2017. The complex relationship between virulence and antibiotic resistance. Genes, 8(1): 39.

Schroth, M. N., Cho, J. J., Green, S. K., & Kominos, S. D. 2018. Epidemiology of Pseudomonas aeruginosa in agricultural areas. Journal of Medical Microbiology, 67(8): 1191-1201.

Senthilkumar, S. R., & Sivakumar, T. 2014. Green tea (Camellia sinensis) mediated synthesis of zinc oxide (ZnO) nanoparticles and studies on their antimicrobial activities. Int. J. Pharm. Pharm. Sci, 6(6): 461-465.

Shah, M. A., & Shah, K. A. 2013. Nanotechnology: The Science of Small. Wiley.

Sharkey, L. K., & O'Neill, A. J. 2019. Molecular Mechanisms of Antibiotic Resistance–Part II. Bacterial Resistance to Antibiotics–From Molecules to Man, 27-50.

Sharland, M., Pulcini, C., Harbarth, S., Zeng, M., Gandra, S., Mathur, S., & Magrini, N. 2018. Classifying antibiotics in the WHO Essential Medicines List for optimal use—be AWaRe. The Lancet Infectious Diseases, 18(1): 18-20.

Shrivastava, S. R., Shrivastava, P. S., & Ramasamy, J. 2018. World health organization releases global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Journal of Medical Society, 32(1): 76.

Skariyachan, S., Sridhar, V. S., Packirisamy, S., Kumargowda, S. T., & Challapilli, S. B. 2018. Recent perspectives on the molecular basis of biofilm formation by Pseudomonas aeruginosa and approaches for treatment and biofilm dispersal. Folia microbiologica, 63(4): 413-432.

Stover, C. K., Pham, X. Q., Erwin, A. L., Mizoguchi, S. D., Warrener, P., Hickey, M. J., & Olson, M. V. 2000. Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. Nature, 406(6799): 959-964.

Sultan, A., Khan, H. M., Malik, A., Ansari, A., Azam, A., & Perween, N. 2015. Antibacterial activity of ZnO nanoparticles against ESBL and Amp-C producing

gram negative isolates from superficial wound infections. Int. J. Curr. Microbiol. App. Sci, 1: 38-47.

Tacconelli, E. 2017. Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development.

Tang, J., Kang, M., Chen, H., Shi, X., Zhou, R., Chen, J. and Du, Y. 2011. The Staphylococcal nuclease prevents biofilm formation in Staphylococcus aureus and other biofilm-forming bacteria. Science China Life Science,54(9):863-9

Tang, P. C., Lee, C. C., Li, C. W., Li, M. C., Ko, W. C., & Lee, N. Y. 2017. Time-to-positivity of blood culture: an independent prognostic factor of monomicrobial Pseudomonas aeruginosa bacteremia. Journal of Microbiology, Immunology and Infection, 50(4): 486-493.

Torok, E., Moran, E., & Cooke, F. 2009. Oxford handbook of infectious diseases and microbiology. OUP Oxford.

Trastoy, R., Manso, T., Fernández-García, L., Blasco, L., Ambroa, A., Perez Del Molino, M. L., & Tomás, M. 2018. Mechanisms of bacterial tolerance and persistence in the gastrointestinal and respiratory environments. Clinical microbiology reviews, 31(4): e00023-18.

Ullah, W., Qasim, M., Rahman, H., Jie, Y., & Muhammad, N. 2017. Beta-lactamase-producing Pseudomonas aeruginosa: phenotypic characteristics and molecular identification of virulence genes. Journal of the Chinese Medical Association, 80(3): 173-177.

Uskokovic, V. 2008. Nanomaterials and nanotechnologies: approaching the crest of this big wave. Current Nanoscience, 4(2): 119-129.

Vandepitte, J., Verhaegen, J., Engbaek, K., Piot, P., Heuck, C. C., Rohner, P., & Heuck, C. C. 2003. Basic laboratory procedures in clinical bacteriology. World Health Organization.

Veesenmeyer, J. L., Hauser, A. R., Lisboa, T., & Rello, J. 2009. Pseudomonas aeruginosa virulence and therapy: evolving translational strategies. Critical care medicine, 37(5): 1777.

Vimala, K., Sundarraj, S., Paulpandi, M., Vengatesan, S., & Kannan, S. 2014. Green synthesized doxorubicin loaded zinc oxide nanoparticles regulates the Bax and

Bcl-2 expression in breast and colon carcinoma. Process biochemistry, 49(1): 160-172.

WHO. 2017. Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis (No. WHO/EMP/IAU/2017.12). World Health Organization.

WHO. 2019. Antibacterial Agents in Clinical Development: an Analysis of the Antibacterial Clinical Development Pipeline, Including Tuberculosis. Geneva: World Health Organization.

Wickremasinghe, H., Yu, H. H., Azad, M. A., Zhao, J., Bergen, P. J., Velkov, T., & Li, J. 2021. Clinically Relevant Concentrations of Polymyxin B and Meropenem Synergistically Kill Multidrug-Resistant Pseudomonas aeruginosa and Minimize Biofilm Formation. Antibiotics, 10(4): 405.

Wijaya, A. 2021. Characterization and targeting of the 2-methylcitrate cycle in Pseudomonas aeruginosa (Doctoral dissertation, University of Cambridge).

Willyard, C. 2017. The drug-resistant bacteria that pose the greatest health threats. Nature News, 543(7643): 15.

Witwit, I. N., Mubark, H. M., Al-Labban, H. M. Y., & Aljanaby, A. A. J. 2019. Synthesis and characterization of new imidazole azo ligand with some of transition metal ions, and their biological effect on two pathogenic bacteria of burn patients.

Wojnicz, D., Tichazek-Goska, D. and Kicia, M. 2015. Entacyclic triterpenes Combined with ciprofloxacin help to eradicate the biofilm formed in vitro by E. coli. Indian journal of medical research, 141(3): 343-353.

Yang, X., Lai, Y., Li, C., Yang, J., Jia, M., & Sheng, J. 2020. Molecular epidemiology of Pseudomonas aeruginosa isolated from lower respiratory tract of ICU patients. Brazilian Journal of Biology, 81: 351-360.

Yeboah, K. O. 2021. Identification of Transcription Regulators of the AlgZ/R Two-Components Regulatory System in Pseudomonas aeruginosa (Doctoral dissertation, East Tennessee State University).

Yusuf, E., Van Herendael, B., Verbrugghe, W., Ieven, M., Goovaerts, E., Bergs, K., & Goossens, H. 2017. Emergence of antimicrobial resistance to Pseudomonas

aeruginosa in the intensive care unit: association with the duration of antibiotic exposure and mode of administration. Annals of intensive care, 7(1): 1-7.

APPENDICES

APPENDIX 1. Questionnaire

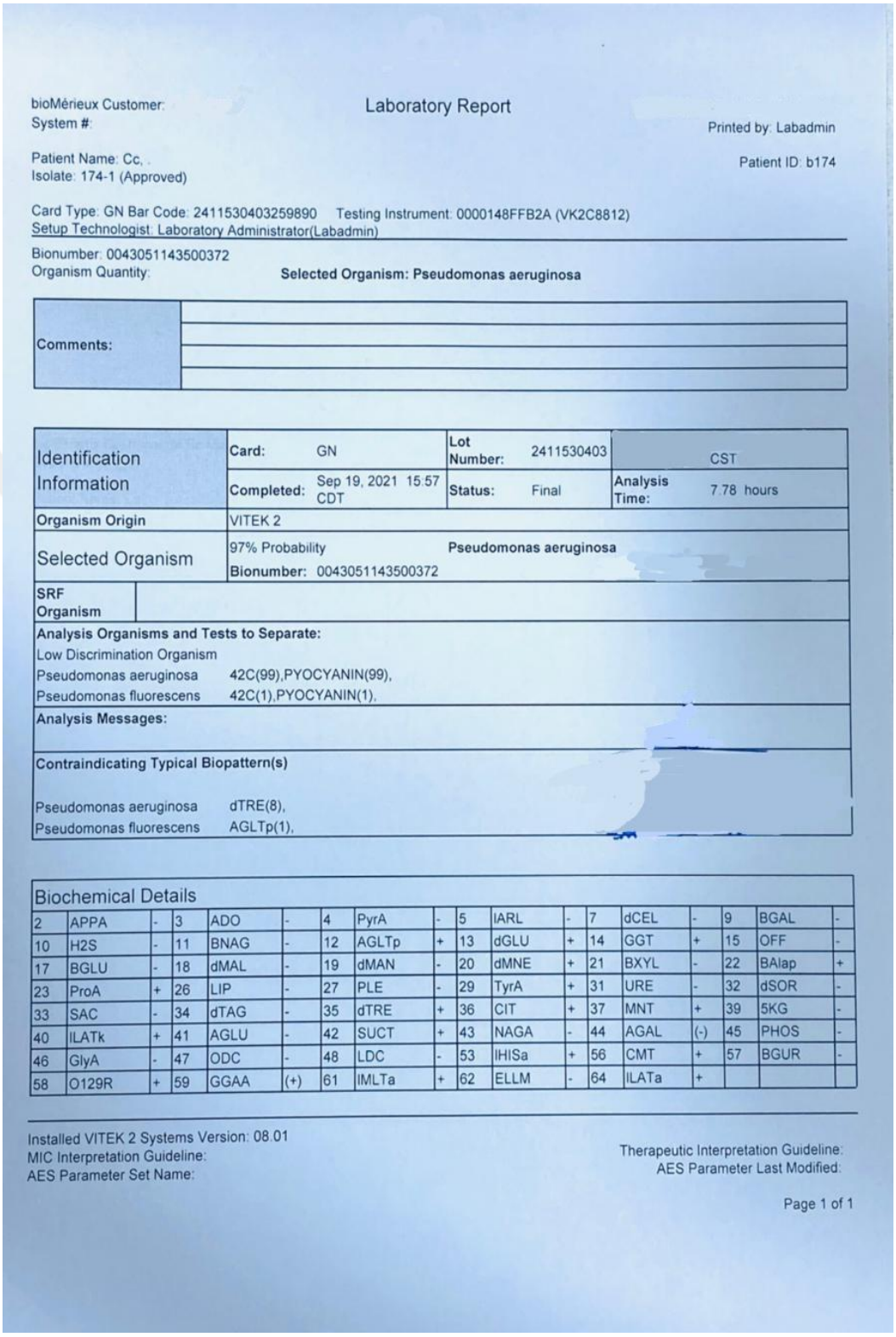
APPENDIX 2. VITEK report

APPENDIX 3. Antibiotic susceptibility

APPENDIX 1. Questionnaire

No. of sample	Name	Age	Gender	Source

APPENDIX 2. VITEK report



bioMérieux Customer:
System #:

Laboratory Report

Printed by: Labadmin

Patient Name: Cc, .
Isolate: 174-1 (Approved)

Patient ID: b174

Card Type: GN Bar Code: 2411530403259890 Testing Instrument: 0000148FFB2A (VK2C8812)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0043051143500372
Organism Quantity:

Selected Organism: Pseudomonas aeruginosa

Comments:	

Identification Information	Card: GN	Lot Number: 2411530403	CST
	Completed: Sep 19, 2021 15:57 CDT	Status: Final	Analysis Time: 7.78 hours
Organism Origin	VITEK 2		
Selected Organism	97% Probability Bionumber: 0043051143500372	Pseudomonas aeruginosa	
SRF Organism			

Analysis Organisms and Tests to Separate:

Low Discrimination Organism
Pseudomonas aeruginosa 42C(99),PYOCYANIN(99),
Pseudomonas fluorescens 42C(1),PYOCYANIN(1),

Analysis Messages:

Contraindicating Typical Biopattern(s)

Pseudomonas aeruginosa dTRE(8),
Pseudomonas fluorescens AGLTp(1),

Biochemical Details

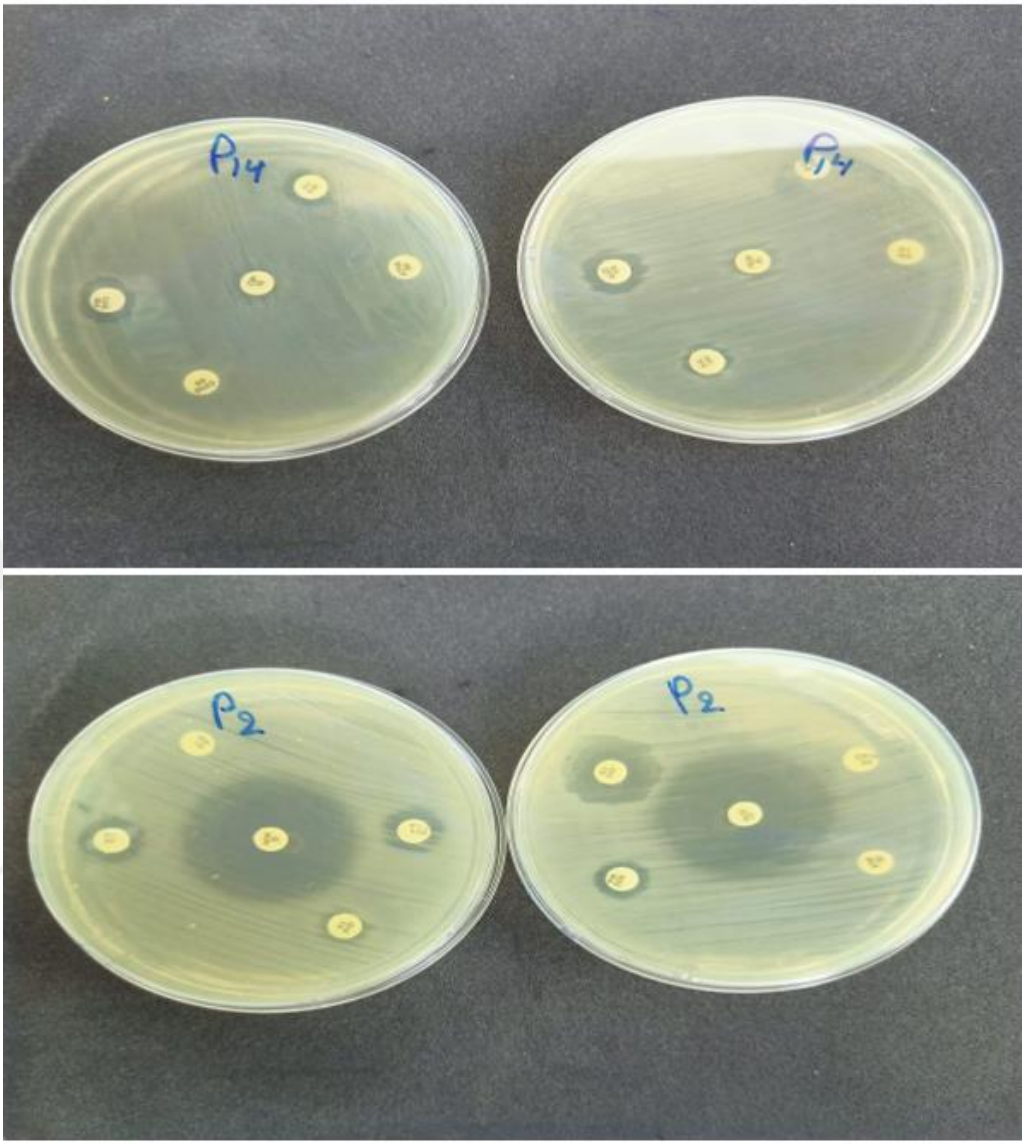
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	+
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	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	+			

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

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Page 1 of 1

APPENDIX 3. Antibiotic susceptibility



CURRICULUM VITAE

Personal Information

Name and Surname : Hiba Adnan ABDULHUSSEIN

Education

MSc	Çankırı Karatekin University Graduate School of Natural and Applied Sciences Department of Biology	2019-Present
Undergraduate	University of Diyala College of Education Department of Biology	2007-2010