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|  | **REPUBLIC OF TÜRKİYE**  **KIRŞEHİR AHİ EVRAN UNIVERSITY**  **INSTITUTE OF NATURAL AND APPLIED SCIENCES**  **DEPARTMENT OF MOLECULAR  BIOLOGY AND GENETICS** |  |

**EFFECT OF NANOPARTICLES ON THE GENE EXPRESSION OF VIRULENCE FACTORS OF *Pseudomonas aeruginosa***

**ALI SULTAN MAALA AL-SHAMMARI**

**MASTER’S THESIS/ DOCTORAL THESIS**

**KIRŞEHİR**

**2023**

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**MASTER’S THESIS / DOCTORAL THESIS**

**SUPERVISOR**

**Asst. Prof. Dr. Lütfi TUTAR**

**KIRŞEHİR**

**2023**

**MASTER’S THESIS APPROVAL**

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**INSTITUTE OF NATURAL AND APPLIED SCIENCES**

**MASTER’S THESIS / DOCTORAL THESIS**

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| October, 2023 | Ali Sultan MAALA AL-SHAMMARI |

# ABSTRACT

**MASTER'S THESIS / DOCTORAL THESIS**

**EFFECT OF NANOPARTICLES ON THE GENE EXPRESSION OF VIRULENCE FACTORS OF *Pseudomonas aeruginosa***

**ALI SULTAN MAALA AL-SHAMMARI**

**KIRŞEHİR AHİ EVRAN UNIVERSITY**

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**DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS**

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| **Jury:** | **Assist. Prof. Dr. Lütfi TUTAR**  **Prof. Dr. Yusuf TUTAR**  **Assist. Prof. Dr. Sevinç AKÇAY** |

*Pseudomonas aeruginosa*, an opportunistic pathogen, is a primary contributor to illness and death among burn patients and individuals with compromised immune systems. There is a growing need for the exploration and advancement of alternative therapeutic approaches that offer fresh avenues to combat *P. aeruginosa* infections. This demand is continuously increasing, with heightened attention directed towards this area of research. This study included One hundred and twenty clinical specimens collected from patients with different infections from four hospitals in Baghdad, which were cultured on Cetrimide agar, Blood agar, and MacConkey agar plates for isolation and identification of P. aeruginosa. According to morphological and biochemical tests, 55 *Pseudomonas aeruginosa* isolates (45.8%) were found in all samples. The prevalence of these isolates was 28 (50.9%) in female patients, compared to 27 (49.0%) in male patients, as shown in table (4-1); the highest rate of bacterial infection was within the age group 31 (20-30 year), followed by 12 (31-40 year), 10 (41-50 year), and 2 (61-70 year) respectively. In the present study, results of biofilm formation by the microtiter plate method showed that ten isolates from 55 isolates (18.2%) were strong technique using the rspL gene as the housekeeping gene of *P. aeruginosa*. All the tested *P. aeruginosa* clinically contained the rspL gene. The results showed that the last gene was found in 80% of isolates that produce strong biofilm, while the rhlI gene was found in all potent biofilm isolates. Ten isolates from 55 isolates were potent biofilm producers, and 8 contained both lasI genes (80%), while rhlI was found in all these isolates. The quantitative PCR reaction experiment involved six highly proficient biofilm producer isolates of *Pseudomonas aeruginosa*, each containing two biofilm genes. These isolates were deliberately selected with varying sub-MIC values to ZnO-np. In this study, the mRNA expression of biofilm genes was examined through a quantitative RT-PCR assay, comparing the samples treated with ZnO-np to those left untreated, using concentrations below the minimum inhibitory concentration (MIC) for each sample during bacterial growth. The results revealed a significant down-regulation in biofilm genes in the present of ZnO-np. The results showed a significant positive correlation between gene expression of RSPL, lasI, and rhlI genes and biofilm formation using Pearson correlation analysis which included all the tested isolates before and after the treatment with ZnO-np.

**Keywords:** *Pseudomonas aeruginosa*, Nanoparticles, Zinc oxide, Virulence, Biofilm.

# ÖZET

**YÜKSEK LİSANS TEZİ /DOKTORA TEZİ**

**NANOPARTİKÜLLERİN *Pseudomonas aeruginosa*'nın VİRÜLANS FAKTÖRLERİNİN GEN EKSPRESYONU ÜZERİNDEKİ ETKİSİ**

**ALI SULTAN MAALA AL-SHAMMARI**

**KIRŞEHİR AHİ EVRAN ÜNİVERSİTESİ**

**FEN BİLİMLERİ ENSTİTÜSÜ**

**MOLEKÜLER BİYOLOJİ VE GENETİK ANABİLİM DALI**

|  |  |
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Fırsatçı bir patojen olan *Pseudomonas aeruginosa*, yanık hastaları ve bağışıklık sistemi zayıf olan kişiler arasında hastalık ve ölüme neden olan birincil faktördür. *P. aeruginosa* enfeksiyonlarıyla mücadelede yeni yollar sunan alternatif tedavi yaklaşımlarının araştırılmasına ve geliştirilmesine yönelik artan bir ihtiyaç vardır. Bu araştırma alanına yönelik ilginin artmasıyla birlikte bu talep sürekli olarak artmaktadır. Bu çalışma, Bağdat'taki dört hastanede farklı enfeksiyonlara sahip hastalardan toplanan ve P. aeruginosa'nın izolasyonu ve tanımlanması için Cetrimide agar, Blood agar ve MacConkey agar plaklarında kültürlenen 120 klinik örneği içermektedir. Morfolojik ve biyokimyasal testlere göre tüm örneklerde 55 adet *Pseudomonas aeruginosa* izolatı (%45,8) tespit edilmiştir. Bu izolatların prevalansı kadın hastalarda 28 (%50,9) iken erkek hastalarda 27 (%49,0) olarak tespit edilmiş; bakteriyel enfeksiyon oranının en yüksek olduğu yaş grubu 31 (20-30 yaş), bunu sırasıyla 12 (31-40 yaş), 10 (41-50 yaş) ve 2 (61-70 yaş) takip etmiştir. Bu çalışmada mikrotitre plak yöntemiyle biyofilm oluşumu sonuçları, 55 izolattan 10'unun (%18,2) güçlü biyofilm üreticisi olduğu bulunmuştur. Ayrıca izolatların 21'inin (%38,2) orta derecede biyofilm üreticisi olduğu, diğer izolatların (n=24; %43,6) biyofilm üretimi açısından zayıf olduğu tespit edilmiştir. deneyi, her biri iki biyofilm geni içeren, *Pseudomonas aeruginosa*'nın altı adet oldukça yetkin biyofilm üreticisi izolatını içermektedir. Bu izolatlar, ZnO-np'ye göre değişen alt MİK değerleri ile bilinçli olarak seçilmiştir. Bu çalışmada, biyofilm genlerinin mRNA ekspresyonu, bakteriyel büyüme sırasında her bir numune için minimum inhibitör konsantrasyonunun (MIC) altındaki konsantrasyonlar kullanılarak ZnO-np ile tedavi edilen numuneler ile tedavi edilmeyen numuneler karşılaştırılarak kantitatif bir RT-PCR tahlili yoluyla incelenmiştir. Sonuçlar, ZnO-np varlığında biyofilm genlerinde önemli bir aşağı regülasyon olduğunu ortaya çıkarmıştır. Sonuçlar, ZnO-np tedavisi öncesinde ve sonrasında test edilen tüm izolatları içeren Pearson korelasyon analizi kullanılarak RSPL, lasI ve rhlI genlerinin gen ekspresyonu ile biyofilm oluşumu arasında anlamlı bir pozitif korelasyon olduğunu göstermiştir.

**Anahtar Kelimeler:** *Pseudomonas aeruginosa*, Nanoparçacık, Çinko oksit, Virülans, Biyofilm

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# LIST OF ICONS AND ABBREVIATIONS

|  |  |  |
| --- | --- | --- |
| **Icons** |  | **Described** |
| *°C* | **:** | Degrees Celsius |
| *µl* | **:** | Microliters |
| *%* | **:** | Percentage |
|  |  |  |
| **Abbreviations** |  | **Described** |
| **AIDS** | **:** | Acquired Immune Deficiency Syndrome |
| **AR** | **:** | Antibiotic-Resistant |
| **CF** | **:** | Cystic Fibrosis |
| **DNA** | **:** | Deoxyribonucleic Acid |
| **cDNA** | **:** | Complementary Deoxyribonucleic Acid |
| **NHS** | **:** | National Health Service |
| **PCR** | **:** | Polymerase Chain Reaction |
| **rRNA** | **:** | Ribosomal Ribonucleic Acid |
| **SCD** | **:** | Special Care Department |
| **ZnO** | **:** | Zinc Oxide |

# INTRODUCTION

*Pseudomonas aeruginosa* is recognized as a significant and versatile pathogen, capable of causing infections in various tissues with varying levels of severity (Lotfpour and Amini, 2020). It is responsible for a wide range of conditions including wounds, burns, urinary tract infections, pneumonia, keratitis, otitis externa, and folliculitis. Furthermore, *P. aeruginosa* poses a considerable threat as a hospital-acquired infection due to its high resistance to antimicrobials and its ability to thrive in nutrient-deprived environments, making eradication challenging (Gellatly and Hancock, 2013). Numerous reports indicate that drug-resistant *P. aeruginosa* infections are associated with significant increases in mortality rates, morbidity, prolonged hospital stays, the need for chronic treatment, and surgical interventions (Ibraheem et al., 2019). *Pseudomonas aeruginosa* is an uncommon disease that primarily affects individuals with multiple underlying conditions such as cancer, AIDS, cystic fibrosis, as well as those with implants or burn injuries. It is a widespread disease that exhibits a propensity for developing antibiotic resistance, thus rendering antibiotic treatments ineffective (Ali et al., 2020). Antibiotic-resistant (AR) infections have become prevalent worldwide, exacerbated by a lack of new antibiotic development in the pharmaceutical industry across many cultures. AR *P. aeruginosa* stands out as a prominent causative agent of healthcare-associated diseases, contributing to global health concerns as multi-drug-resistant strains continue to emerge. The expression of impermeable proteins in the outer membrane is immune to most antibiotics (Samrot et al., 2018). *P. aeruginosa* also uses various mechanisms to survive antibiotics, including seclusion of inactivating enzymes, expression of efflux pumps, and chromosomal mutations (Hemeg, 2017).

Biofilm formation by this species increases the high resistance to antibiotics, including fluoroquinolones, beta-lactams, and carbapenems, and this barrier decreases the possibility of bacteria penetrating the immune cell of biofilms and of antibiotics and acts as adequate protection against the host immune systems and antibiotic agents, which leads to continuous colonization of the organism. Treating burn wound bacterial pathogens is a significant challenge, and new methods of reducing death rates associated with bacterial infections in burn injuries are needed (Shariati et al., 2019). The emergence of resistance in treatment, which is proved to duplicate the time of hospitalization and total cost of patient care, is even more troublesome (Jindal et al., 2015).

Many previous studies exhibited the nanoparticles' applications in the medical field and the treatment of infectious diseases. Higher efficiency was reported on metal oxide nanoparticles resistant strains, such as zinc oxide (ZnO) and silver. Incredible antimicrobial activity and a significant reduction in skin infections and inflammatory function of mice were found in ZnO nanoparticles (Sirelkhatim et al., 2015).

The *Pseudomonas aeruginosa* problem is very severe in local hospitals in Iraq. The current study aims to recognize the effect of ZnO nanoparticles on biofilm formation as a virulence factor of *Pseudomonas aeruginosa* isolates, to eradicate these bacteria by seeking alternative antimicrobial materials.

# LITERATURE REVIEW

In this section, relevant literature on the subject is presented.

## 2.1. *Pseudomonas aeruginosa*

*Pseudomonas aeruginosa* is categorized as a lactose-non-fermenting, gram-negative, non-spore-forming, and motile by one polar flagellum bacillus. The majority of isolates are catalase and oxidase positive. It can use aerobic respiration as its preferred mode of metabolism because it is an obligate respiration. However, it can also breathe anaerobically using nitrate or other alternate electron acceptors; thus, it is the reason why bacteria is common throughout the planet and can be found in water, soil, or sewage as well as in plant, animal, or human hosts (Noomi, 2018). An opportunistic pathogen, *Pseudomonas aeruginosa*, can infect people with acute or chronic illnesses. It frequently infects persons with weak immune systems, and infections like this have a high death rate in burn victims or people who require mechanical breathing. It is also crucial for patients with persistent respiratory infections, such as cystic fibrosis (CF) and other respiratory system illnesses (Schubiger et al., 2020). When burns or wounds occur, the skin acts as a natural barrier, safeguarding the body's tissues. However, this creates an ideal environment for the proliferation of microorganisms, including bacteria such as Pseudomonas spp., posing a significant threat to patients with burns and wounds. These bacteria can infiltrate the bloodstream, leading to bacteremia and septicemia, particularly affecting individuals with leukemia and immunodeficiency (Jalil et al., 2018). Notably, these bacteria possess remarkable resistance to disinfectants, contributing to their role in hospital-acquired infections. They have been found to thrive in solutions, disinfectants, and detergents containing hexachlorophene, thereby further complicating infection control efforts (Sudhakar et al., 2015). It can be challenging to treat this pathogen because of its natural and acquired antibiotic resistance. It has an extraordinary ability to acquire antibiotic-resistance genes, spread from patient to patient, and survive in the hospital setting (Lila et al., 2018).

*Pseudomonas aeruginosa* can grow in the laboratory on MacConkey agar medium, and their colonies are pale due to their inability to ferment lactose sugar and smell like fermented grapes. On blood agar, colonies appear dark in color and surrounded by a transparent area, indicating their ability to hemolyze blood (type -Hemolytic) due to hemolysin production, as well as their ability to grow at 42 °C (Hossain et al., 2013). *P. aeruginosa* has the cytochrome oxidase enzyme, a phylogenetic taxonomic feature, and many members produce pigments. They do not form spores and can produce pigments that fluoresce, such as pyocyanine (green-blue) and pyoverdin (yellow-green) (Bunyan et al., 2019). *P. aeruginosa* creates various virulence factors necessary to bypass the host immune system and other natural defenses. One of P. aeruginosa's most crucial virulence factors is the development of biofilms (Pachori et al., 2019).

## 2.2. *Pseudomonas aeruginosa* Classification

In 1882, Gessard was first isolated from purulent wounds by *Pseudomonas aeruginosa*, after which it became known as *Pseudomonas pyocyanin* and then as *Pseudomonas aeruginosa*, and was called *Bacillus pyocyanin* (Oliveira and Reygaert, 2022). The name of the bacteria, Pseudomonas, originates from the Greek word 'pseudo,' meaning false or fake, and the second Greek term refers to copper rust (Brooks et al., 2010). *Pseudomonas aeruginosa* is part of the Pseudomonadaceae family and includes several species in the Pseudomonas genus (Bailey et al., 2014). The classification of these bactéries is according to the following graph (Slonczewski and Foster, 2014) and is based on DNA sequence, especially Sequence 16S rRNA (Tripathi et al., 2013).

## 2.3. General Characteristics of *Pseudomonas aeruginosa*

The gram-negative bacteria appear in *Pseudomonas aeruginosa* as a single rod form, pairs, and short chains (Oliveira and Reygaert, 2022). Bacteria of *P. aeruginosa* are slightly or directly curved, stained rods with a diameter of 0.6 to 2.0 μm; motile by a single or more polar flagellum and non-spore-forming (Govan, 2007). *P. aeruginosa* is an obligate aerobic bacterium that demonstrates robust growth on diverse culture media and sometimes releases a distinct odor resembling a sweet grape or corn taco aroma. Blood is hemolyzed by certain strains. *P. aeruginosa* colonies are smooth, round, and pigmented (Oliveira and Reygaert, 2022).

*P. aeruginosa* is an oxidase-positive, frequently pigmented, and unable to degrade carbohydrates, but many strains oxidize glucose without gas formation (Brooks et al., 2013). In the existence of arginine or nitrates as terminal electron accepters, anaerobic growth from *P. aeruginosa* bacteria has also been proved possible (Todar, 2008). It grows well at 37-42°C, not 4°C, and grows at 42°C; it helps to distinguish *P. aeruginosa* from another fluorescent pigment-producing Pseudomonas. Some Pseudomonas species can be grown at 45°C (Fothergill et al., 2007).

## 2.4. *Pseudomonas aeruginosa* Pathogenicity

*P. aeruginosa* is one of the primary nosocomial infections in the hospital setting (Poole, 2011). In patients with severe medical conditions, it may lead to numerous acute opportunistic infections (Gellatly and Hancock, 2013).

Infections are more frequent and diverse during hospitalization for those with immuno-depression, extreme burns, wounds, chemotherapy, and acquired immune deficiency syndrome (AIDS). The most at risk are those with immuno-depressed patients (Park et al., 2014).

*P. aeruginosa* infections are usually immune to several antibiotics, which can lead to severe and recurrent infections (Doosti et al., 2013); this leads to secondary fungal infections and other complications, a longer stay in the hospital, medication failure, and premature death in cystic fibrosis patients (Tan et al., 2014). *P. aeruginosa* is a pathogen commonly linked to nosocomial pneumonia, nosocomial urinary tract infections, surgical site infections, severe burns, wound infections, external otitis, keratitis, and folliculitis (Gellatly and Hancock, 2013). Either neoplastic disease chemotherapy or broader spectrum antibiotic therapy infections of patients (Shaan and Robert, 2013).

Via pathogenesis of P. aeruginosa, virulence factors may lead the human host to infection at various stages at the same time or act independently (Wolska et al., 2011), i.e., lipopolysaccharide, alginate (exopolysaccharide), which directly influences fever, shock, oliguria or leukocytosis, Leukopenia, adult condition of respirable discomfort and intravascular clotting, pili and flagella, and secreted virulence factors, like toxins, enzymes like protease, phospholipase, elastases and other small molecules such as rhamnolipid, phenazines and cyanide inducing or interacting with the host immune response to trigger infection (Rafie et al.,2014).

When *P. aeruginosa* finds a proper place for colonization, they begin to express the virulence factor genes and eventually activate the infection procedure, followed by the development of virulence factor in the host cells (Rasko and Sperandio, 2010).

The creation of the diversity of pseudomonas different adaptation processes such as diet and metabolic Pathways in addition to gene expression regulation (Riera et al., 2011). Pseudomonas' ability to build biofilms. Aeruginosa contributes significantly to its virulence in the catheter lumen and the lung of cystic fibrosis patients. Pseudomonas Immune to specific antimicrobial agents, aeruginosa becomes dominant as more susceptible bacteria in the normal microbiota are eliminated. This resistant is due to the capacity of biofilms of bacteria that are embedded in the bacteria in the exopolysaccharide matrix (Salman et al., 2019).

*P. aeruginosa* provides pathogenesis and establishes quorum sensing multidrug resistance. The presence of MDR pseudomonas has been reported to date. In essential hospital terms such as Burn Unit and Special Care Department (SCD), aero xylic strains are essential in preventing MDR-infected infections (Mahnaie et al., 2020). Longitudinal studies, including longitudinal methodology, provided details on genetic changes subject to *P. aeruginosa* and permitted comparing particular expression genes in various patient periods (Hussien et al., 2012).

Gene expression changes have been identified in multidrug efflux pumps, quorum sensing regulators, and alginate biosynthesis mutations (Rezaie et al., 2018).

## 2.5. *P. aeruginosa* Infections

In this section, the topics of respiratory diseases are discussed.

### 2.5.1. Respiratory tract infections

In patients with immunosuppression, chronic lung disease, and heart failure causing Pneumonia, aeruginosa is a primary and joint cause of respiratory tract, and acute respiratory infections have frequent aeruginosa—silver cells. Bronchiectasis is also caused by aeruginosa. Mucoid *P. aeruginosa* strains are common and hard to treat in patients with cystic fibrosis (Wuerth et al., 2019).

### 2.5.2. Skin infections

*P. aeruginosa* is a common cause of thin- and hair follicles, skin infections, and damage. This type of infection is transmitted by water using water pools, mineral water baths as well as by skin contact, since it makes it possible to penetrate hair follicles and then begins the production of inflammatory toxins in areas such as rabbits and armpits as well as areas of the uniforms worn by swimmers, as this is the most common type of infection (Domenico et al., 2017). Skin lesions are difficult to treat, although different types of antibiotics with a wide power threaten the patient's treatment (Nagoba et al., 2013).

### 2.5.3. Ear infections

*P. aeruginosa* is a common cause of chronic otitis media and other ear inflammations, which also can lead to external otitis, including malignant otitis externa (Roland and Stroman, 2002). Here, the patient develops a hole in the eardrum membrane because of the entry of the pathogen into the water through the pharyngeal nose or the hole when bathing or swimming (Cole et al., 2014).

### 2.5.4. Urinary tract infections (UTI)

Bacteria entering the urinary tract through catheters, instruments, and irrigation solutions is one of the most frequent *P. aeruginosa* infections. It is the leading cause of UTI in the hospital; it is more common in men, particularly during pregnancy and birth. This injury occurs (Cole et al., 2014; Cole and Lee, 2019).

### 2.5.5. Infections of burns and wounds

Wound infection poses a significant risk for patients with burns, and burn wounds caused by *P. aeruginosa* are particularly challenging to treat within hospital settings (Jault et al., 2018). Burn injuries are highly debilitating and can have long-lasting impacts on a patient's health. Globally, an estimated 265,000 deaths per year are directly attributed to fire-related burns, with 90% of burn incidents occurring in developing nations where patient mortality rates can reach 100%. These burns often cover more than 40% of the total body surface area. While millions of people worldwide suffer from burn-related conditions, the National Health Service (NHS) in the United Kingdom alone sees approximately 90,000 hospital admissions annually due to burns (Guest et al., 2020).

## 2.6. *Pseudomonas aeruginosa* Epidemiology

These bacteria spread across the atmosphere and live in the soil, marshes, and aquatic habitats. They live on plant and animal fabrics. These bacteria can thrive in disinfectants, liquid medical products like sabyns drop in the eyes, saline solutions, anesthetics, and many other medical products; these bacteria have their survival skills (Stover et al., 2000).

*P. aeruginosa* can live in less healthy environments and sustain various physical conditions that allow bacteria to exist in hospital and community settings (Lister et al., 2009). It can replicate and settle efficiently in the tissues affected, leading to systemic sepsis and increasing death (Diggle and Whiteley, 2020). Metabolic flexibility and high genetic characteristics allow this bacteria to adapt to the most artificial and natural environments worldwide, from medical equipment, animal and plant tissues, water, soil, and even the ISS (Kim et al., 2013).

A high percentage of these bacteria will occur within 72 hours in a hospital. It requires an immune suppressive agent, anti-metabolism, and radiation, which help to raise infections and spread from these bacteria called nosocomial infections in patients with severe burns and wounds. Using infected surgical equipment promotes the spread of this organism and direct and indirect communication between patients (Japion et al., 2009). These *P. aeruginosa* infections also cause death and morbidity because they establish antibiotic resistance rapidly and adapt quickly to different environments and cell-related and extracellular virulence factors (Mitov et al., 2010).

## 2.7. Virulence Factors of *Pseudomonas aeruginosa*

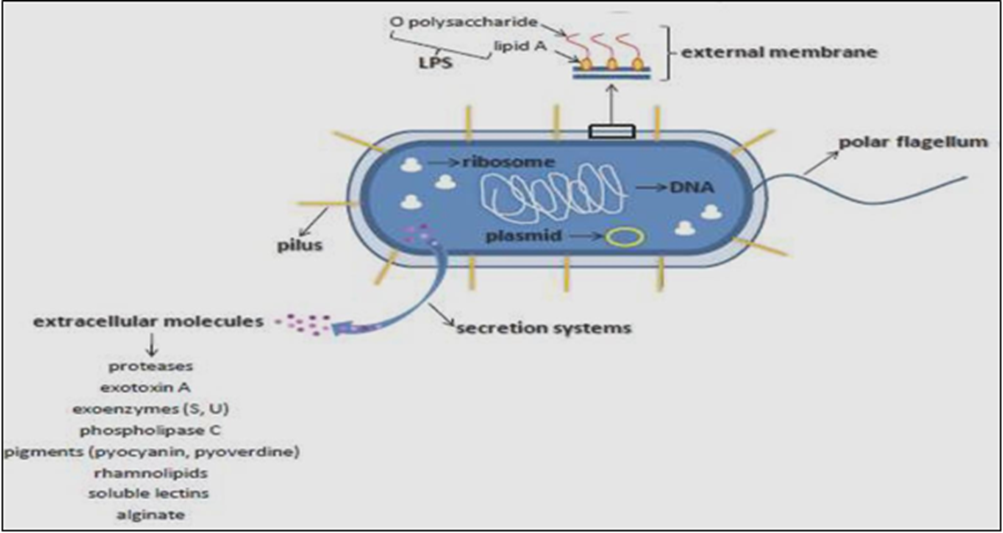
*P. aeruginosa* has several factors of virulence that allow it to colonize within the body of its host organism, leading to illnesses, enzymes, and exotoxins, for instance. It is as well a biofilm that defends against phagocytes and environmental stress. (Macin and Akyon, 2017).

It will be necessary to make extracellular enzymes, including elastases, proteases, exotoxin A, and hemolysins, which cause cell death or interfere with the host's immune response to the disease, which are the most.

Significant virulence factors include biofilms and pili, exoenzymes like S, T, U, and Y. (Karen et al.,2016).

Pyocyanin is the most critical, non-fluorescent pigment (blue soluble in water), and pyoverdine is fluoresced pigment (green-yellowish, also known as Pseudobactin), which is toxic to host cells. This kind of bacteria produced a variety of pigments that inhibited the growth of other bacteria. It is also a red pigment, and Pyomelanin is a brown or black pigment, for example, Pyorubin (Lee et al., 2014).

Many antibiotics may become resistant to *P. aeruginosa*, making treatment for their infections more difficult. Biofilm bacteria can create bacterial communities within an exopolysaccharide matrix, which gives them resistance. This environmental bacterium can form biofilms on surfaces such as CF lungs, contact lenses, and infected catheters, both surface and non-living (Hoiby et al., 2010). Protein, polysaccharides, and extracellular DNA are used for exopolysaccharides (Storz et al., 2012). It holds together the cell that is required to interact with the cell. It also enables the formation of 3D structures, providing bacteria with increased access to nutrients and multicellular livelihood advantages (Sharma et al., 2014).



**Figure 2.1.** *Pseudomonas aeruginosa* virulence factors (Galdino et al., 2017)

### 2.7.1. Biofilm formation

The biofilm consists of colonies of exopolysaccharide formed by the *P. aeruginosa*, which are surrounded by microorganisms (Tseng et al., 2019). The essence of biofilm is hydrophilic, and water constitutes approximately 97% of membranes. Biofilm has rivers that it uses for the transportation of nutrients. In extreme, challenging environmental conditions, Biofilm is responsible for preserving water and nutrients (Wei and Ma, 2013).

Stages of biofilm formation:

Biofilm training stages:

1. Early-stage substratum attachment.

2. The second level of microcolony development and reproduction and accumulation.

3. Separating the microcolonies from the third stage to the various compositions and maturing.

4. The fourth stage of release, distribution, and colonization of the cells outside to new areas (Harper et al., 2014).

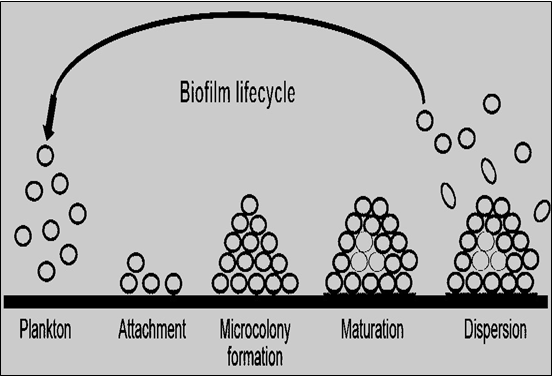


Figure 2.2. Biofilm formation steps (Santos et al., 2018)

However, *P. aeruginosa* 's biofilm formation allows it to remain alive on dry surfaces long. Much research will concentrate on the production phase of biofilm as it is a significant cause of many diseases, such as chronic pneumonia, chronic bladder inflammation, bone inflammation, bacterial cell build-up endocarditis, and toxins (Lanter et al., 2014).

Using Qurum-Sensing genes, the regulation of biopsychological formation can be carried out, and the bacterial cells can thus interact within biofilms (Li and Tian, 2012).

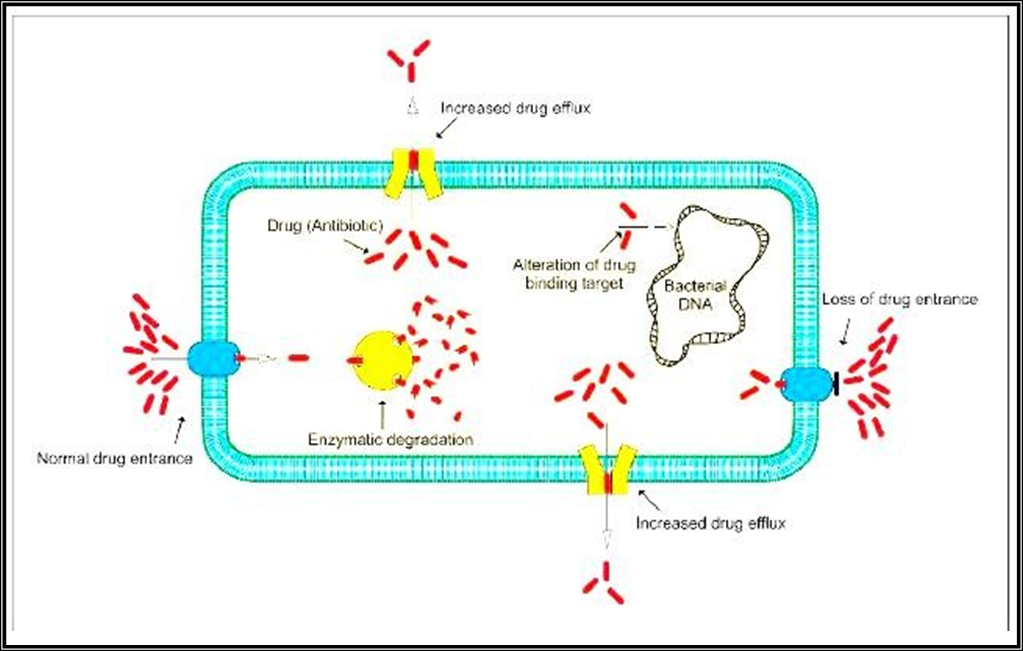
## 2.8. Antibiotic resistance

*P. aeruginosa* has become a significant and commonly occasional nosocomial pathogen. This organism has inherent resistance to many antimicrobial types, resulting in difficult-to-treated infections associated with severe disease and mortality (Obritsch et al., 2004).

The pathogen *P. aeruginosa* presents a significant therapeutic challenge when it comes to antibiotic treatment due to its prevalence in both nosocomial infections and community-acquired diseases. Therefore, the careful selection of appropriate antibiotic dosages is crucial to prevent the development of complications (Lister et al., 2009). *P. aeruginosa* is very resistant to numerous different antibiotics intrinsically. In addition, these bacteria can also quickly develop strong resistance under specific selective pressure either by transferring the resistance genes horizontally or by mutating the chromosomally encoded genes. Bacterial infections are often difficult to treat due to their multidrug resistance phenotype (De Francesco et al., 2013).

*P. aeruginosa* has multiple resistances to antibacterial agents because of different factors, the lowest of which is the low permeability of the cellular bacterial surrounds genetics. It shows a wide variety of antibiotic resistance mechanisms and a P. aeruginosa's ability to acquire other resistance genes from other bacteria through the conjugation, transformation, and transmission of mobile genes (plasmids, transposons, phages) (Tam et al., 2010).

Several *P. aeruginosa* resistance infections have been commonly found in cancer patients, immunocompromised AIDS, COPD, cystic fibrosis, and even by people with diabetes as secondary infections (Hogardt and Heesemann, 2013), leaving serious, high-mortality, blood-stream infections, and healthcare costs to the patient. Several types remain effective agents of *P. aeruginosa* infections (Ceftazidime, Cefepime, Carbapenems, Tobramycin, and Amikacin). Many other antibiotics related to different groups, like Carbencillin, have become more resistant to *P. aeruginosa* isolates (Black, 2012). Carbapenem classes, on the other hand, have good anti-microbial activity, but the propagation and development of acquired carbapenem class resistance is a challenge for control success and therapeutic efforts. The special imipenem carbapenem classes are used extensively in a clinical setting (Riera et al., 2011).



**Figure 2.3.** *Pseudomonas aeruginosa* mechanism for antibiotic resistance (Rocha et al., 2019).

### 2.8.1. Carbapenems resistance

Beta-lactam is the most common antibiotic used for carbapenems (meropenem and imipenem) (Morita et al., 2014). It is an effective agent for treating many Pseudomonas infections because of its beta-lactamase resistance (Ding et al., 2018). The mechanisms of carbapenems antibiotics are penicillin-bound protein inhibition, which rests on the plasma membrane's exterior surface. The bacterial external membrane of carbapenems antibiotics may pass through the porine canals (OprD) (Ocampo-Sosa et al., 2012). Pseudomonas. aeruginosa to low-expression carbapenems, especially metal beta-lactamase and extreme AmpC beta-lactamases, or extreme expression efflux pumps, in outer membrane proteins (OprD) or carbapenemase (Patel and Bonomo, 2013).

### 2.8.2 Extended spectrum beta-lactamase (ESBLs)

Extended-spectrum beta-lactamase in *P. aeruginosa* has already been reported and is remarkably resistant to various antibiotic types, such as penicillins and cephalosporins. ESBLs are new beta-lactamases, which convey a resistance in particular to cephalosporins, some of the latest beta-lactamase antibiotics. Bacterial plasmid genes, which carry genes responsible for resistance to various antimicrobial substances such as Aminoglycosides, Tetracyclines, and Sulfonamides, encode for Extended-Spectrum Beta-Lactamases (ESBLs). These ESBLs have evolved from earlier beta-lactamase enzymes like TEM, SHV, and OXA, with a narrower range of activity in terms of the antibiotics they can degrade. ESBLs contribute to the multidrug resistance observed in gram-negative bacteria. The ESBL enzymes are further categorized into two classes, namely Class A and Class D, based on their structural characteristics (Omer et al., 2020). In *P. aeruginosa* tension, ESBL animatics are mainly observed for the PER classes and PME (class A). The class is called extended-spectrum Class D βlacktamases(ES-OXAs) and are primarily βlactamases from PER, GES, VEB, BEL, and family PME (class A). Antimicrobial resistance is an increasing clinical problem and a recognized global threat to public health.

*P. aeruginosa* exhibits a particular propensity to resistance development. The development of *P. aeruginosa* resistance also limits future treatment choices and is linked to higher mortality and morbidity rates and increased costs (Kumar et al., 2020).

### 2.8.3. Metallo beta-lactamases (MBLs)

The synthesis of metallo-beta-lactamase is the prevailing mechanism of carbapenem resistance. Metallo-beta-lactamase, which belongs to Ambler Class B enzymes, is capable of hydrolyzing beta-lactam antibiotics, including carbapenems. Beta-lactamases use serine as an active site so that beta-lactamase inhibitors such as clavulanic acid or sulbactam eight can be easily degraded. However, MBL-producing Pseudomonas now appears as a nightmare for doctors. The MBL-producing Pseudomonas is a doctor's nightmare. In addition, MBL resistance is situated in a highly mobile genetic element, which allows easy dissemination from patient to patient or from patient to the medical provider. Therefore, preventing MBL Pseudomonas is always better than treating them (Mukherjee et al., 2020).

## 2.9. AmpC Cephalosporins

The wild-type strain of *P. aeruginosa* possesses AmpC cephalosporins that are not inhibited by BLI-inducing inducible molecular class C inhibitors such as clavulanic acid, tazobactam, and sulbactam (Sligl et al., 2015). The expression of AmpC cephalosporins is typically low, providing inherent resistance to aminopenicillins, as well as in combination with BLI, first and second-generation cephalosporins, cephamycins, two third-generation cephalosporins (cefotaxime and ceftriaxone), carbapenems (including ertapenem), and other aminopenicillin-resistant strains, along with reduced membrane permeability and multiple efflux systems (Kumar et al., 2020). However, the wild-type strain of *P. aeruginosa* remains susceptible to carboxypenicillins, ureidopenicillins, C3G ceftazidime, C4G cefepime, aztreonam, imipenem, meropenem, and doripenem, including carbapenems. However, over-expression and point mutation of induced or constitutive AmbCs can offer reduced sensitivity to all β-Lactamine classes except carbapenems. In contrast to the AmpC of Enterobacteriaceae, cefepime can also be affected by AmpC of *P. aeruginosa* and can be produced in serin β-lactamases of Amber Class A of type TEM (Bush 2b), PSE or CARB (carbecillinase) (Lister et al., 2009).

These enzyme substrates are mainly carboxypenicillin and ureidopenicillin and may sometimes be resistant to BLI. These enzymes exhibit different levels of susceptibility to cefepime, cefpirome, and aztreonam, but ceftazidime and carbapenem retain their efficacy against *P. aeruginosa* strains possessing these types of β-lactamases (Gómez et al., 2019).

## 2.10. Quorum sensing (QS) system

QS Systems found in some bacteria can be defined as a cell-to-cell communications system for chemical mediation to co-ordinate gene expression and community group activities. Dr. Peter Greenberg first found QS in the bioluminescent bacterium Vibrio fischeri 1994 (Yang, 2009). Quorum sensing is significant prevalence of many different Species in the bacterial realm can be considered as "speaking" systems that play a significant role in controlling virulence factors (Yin et al., 2012).

An extensive range of virulence factors, including the corresponding effector protein such as extracellular phospholipases, proteases, and type III secreted toxins (Exo U, S, T, and Y) and secreted type II and III systems, have been identified for *P. aeruginosa*. Moreover, Type IV pili and flagella for adhesion and motility of the host cells (Feinbaum et al., 2012). The factors in the virulence enable and facilitate the invasion of the host by bacteria, The evasion of the host's immune system and suppression of the host's immune response are critical aspects of bacterial pathogenicity. While virulence factors undoubtedly contribute to bacterial growth, they do not solely determine it (Chakravarty and Massé, 2019). Quorum sensing (QS) is responsible for regulating the expression of genes associated with many virulence factors, including those involved in toxin production such as hydrogen cyanide (Feinbaum et al., 2012). It also helps regulate wide-ranging community behaviors, including swimming, twitching, and conjugating (Rutherford and Bassler, 2012). All activities rely on the quorum sensing (QS) system, which involves the synthesis, secretion, and detection of molecules called autoinducers (AI) (Kalia and Purohit, 2011). Quorum sensing enables bacteria to assess the local population and make collective decisions based on cell density, thereby coordinating the behavior of the entire bacterial community. These behaviors are usually always associated, but not involved in bacterial viability, with pathogenicity/virulence (LaSarre and Federle, 2013).

Preventing bacterial adherence to different surfaces was one strategy for inhibiting biofilm formation. The surfaces of antimicrobial

Agents, like metallic nanoparticles, can be covered. Quaternary ammonium salt or other surfactants (e.g., QAS) surfactants (Stone et al., 2020)

The standard QS system includes three components:

·The signal molécules (AI) or the Acyl homocerin lactones (AHL) Signal molecules.

- The AI-producing synthase is capable of activating the receptor through the transcription of specific genes responsible for AI biosynthesis (genes encoding the synthase), (Miller and Bassler, 2001).

-The transcriptional regulator and the receptor.

-The QS controls the manifestation of extracellular and cellular associated VFs by producing QS signal molecules in response to population density (AHLs).

Includes the homoserine lactone ring that differs from the length of the N-linked acyl side chains and C3 substitution. The accumulation of AHLs over a threshold level causes its interaction with the LuxR activator family to allow the transcription of the target genes (Steindler and Venturi, 2007).

In a sparsely populated community, the AHLs (acyl-homoserine lactones) secreted into the surrounding medium become highly diluted through diffusion, resulting in minimal activation of the receptor. However, as the density of bacterial cells increases, the AHLs reach a threshold level, triggering receptor activation (Miller and Bassler et al., 2001).

## 2.11. Las system

The LasI gene-producing system leads to a synthesis of L-L-3-oxo- dodecanoyl) homoserin lactone (3-Oxo-C12-AHL) (Smith and Iglewski, 2003).This protein can only bind DNA multimerically and regulate the transcript of several genes at high cell density. LasA (lasA protease)

Expression, apr (alkaline protease), toxA (exotoxin A), and lasB (elastase) are regulated in the system (Siechnela et al., 2010).

### 2.11.1. Rhl system

The rhlI gene is responsible for directing the synthesis of N-(butanol)-L-homoserine (C4-AHL), which interacts with the RhlR regulatory system and activates promoters of target genes (Smith & Iglewski, 2003). The expression of various genes, including alkaline protease, elastase, cyanide, rhamnolipid, and pyocyanin production, is regulated by this system (Dekimpe and Deziel, 2009; Karatuna and Yagci, 2010). Analysis of QS signal molecules may be helpful in various bacterial infections. The detection and identification of QS signals in molecules can provide insight into the density and type of population of the infectious pathogen and its virulence components. Moreover, the new target of developing innovative infection control strategies is the Q of S regulation mechanisms (Rasmussen and Givskov, 2006). Identifying possible drug target factors to combat *P. aeruginosa* infection of AHLs is also essential in clinical environments (Bjarnsholt and Givskov, 2007).

## 2.12. Nanomaterials

### 2.12.1. Background

The word "nano" derives from the Greek word "nano," which means "dwarf," which indicates about 10-9 nm of stuff measuring one trillionth. Due to the broad potential applications such as "nanomedicine" and the unique characteristics of various fields, the nanostructures have attracted more attention and provided simple technology to prepare and synthesize the nano-sized metal particles (Jeevanandam et al., 2018).Nanomaterials (NMs) and nanoparticles (NPs) offer potential solutions to environmental and technological challenges, with their unique characteristics influenced by factors such as composition, size, shape, size range, and distribution (Płaza et al., 2014). The field of nanotechnology is rapidly emerging, finding applications in various scientific and technological domains for creating new substances at the nanoscale. Presently, nanotechnology extends beyond electronics and encompasses diverse areas, including wound healing, anti-inflammatory effects, catalysis, magnetism, and optical and analytical applications (Ali et al., 2020).

### 2.12.2. The inorganic NPs

This group includes magnetic NPs, noble metal NPs, and semi-conductor NPs (gold and silver NPs) (zinc oxide and titanium oxide). Inorganic NPs are increasingly interested because they offer superior material properties for functional versatility. The most outstanding biomedical agents synthesizing NPs are metallic nPs, e.g., zinc, gold, carbon, silver, titanium, iron, palladium, fullerenes, and copper. For increasing environmental impacts, biological approaches and other green summaries must be developed (Vadlapudi and Kaladhar, 2014). Certain NPs are attractive biological marker samples because:

BETHER Small size from 1-100 nm.

-The wide volume-ratio surface.

-Trees' Biological and chemical characteristics, especially protein components, concerning shape and size, and strong affinity to the target.

-The structural robustness despite the granularity of the atom.

-Enhance or delay aggregation of particles depending on the surface type and photo-emission improvement.

-High heat, electrical conductance, and enhanced catalytic surface activity (Sahayaraj and Rajesh, 2011).

### 2.12.3. Nanomaterials as antimicrobials

Traditional antibiotic medicines reduce their ability to deal with common infectious diseases and are necessary for many treatments. Antibiotics have paved the way for unexpected medical and societal development in all healthcare systems. Multi-drug resistance among many pathogenic bacteria in the global market has increased considerably, resulting in the most frequently ineffective antibiotics in controlling infectious diseases, creating an issue in healthcare (Laxminarayan et al., 2013).

Developing new antibacterial systems before drug-resistant pathogens thus represents a significant threat to successful microbial disease treatment (Huttner et al., 2013).

In addition, a nanotechnology is an approach to the development of new antibodies known as nano antibiotics which are efficient in the treatment of infectious diseases, and which have many advantages over traditional antibiotics, including lack of adverse effects, increasing drug-resistant species efficiency, and overcoming the development of resistance, interfering by various biological pathway.

Nano antibiotics exhibit antimicrobial activity either on their own or by enhancing the effectiveness and safety of traditional antibiotic administration, leading to elevated local concentrations (Hajipour et al., 2012). Antimicrobial nanoparticles offer clear advantages, including low toxicity, the ability to overcome resistance, and lower costs compared to conventional antibiotics (Huh and Kwon, 2011).

Antimicrobial NPs may cause the bacterial membrane to disturb mechanically and even "unrecognized" cells as a defense treatment for bacteria (Guzman et al., 2012).

### 2.12.4. Mechanisms for killing bacteria in such NPs include

The production of H2O2, O2 ̄ and OH ̄ reactive oxygen (ROS) species. The bacterial wall membrane cell disorder. DNA synthesis inhibition and activities of intracellular enzymes. Disrupt energy transduction (Xie et al., 2011). Today many antibacterial NM and NPs are being produced to fill the gap in antibiotic treatment failure with the emergence of nanotechnology (Beyth et al., 2015).

### 2.12.5. Zinc oxide nanoparticles (ZnO NPs)

ZnO-np, a well-known nanoparticle, belongs to the category of metal oxide nanoparticles. It is an inorganic compound consisting of zinc oxide, appearing as a white powder that is nearly insoluble in water. ZnO-np finds diverse applications, including antimicrobial treatments, wound healing, UV protection, high catalytic and photochemical functioning. What makes it particularly remarkable is its unique combination of features such as minimal impact on human and animal cells, bacterial toxicity, plasma hydrogen stability, and affordability (Xie et al., 2011). The antibacterial effects of ZnO-np involve the production of reactive oxygen species, which can damage bacterial membranes. Additionally, the generation of hydrogen peroxide and Zn2+ ions has also been found to play a significant role in the antibacterial activity of nanoparticles. However, the development of biofilms can provide protective mechanisms for pathogenic microorganisms against inhibitory compounds (Abdelraheem and Mohamed, 2021).In recent years, nanoparticles with metal oxide properties, such as zinc oxide, have garnered significant attention due to their stability and ability to withstand harsh environmental conditions. These nanoparticles can be easily synthesized at low temperatures using a reflux digestion process and are considered safe for both humans and animals. Zinc compounds are now included in the Generally Recognized as Safe (GRAS) list by the US Food and Drug Administration, indicating their recognized safety (Ali et al., 2020).

ZnO NPs (Zinc Oxide Nanoparticles) induce elevated levels of reactive oxygen species (ROS) and malondialdehyde in bacterial cells, leading to lipid peroxidation of the cell membrane. Transmission electron microscopy images of treated bacterial cells confirm that ZnO NPs disrupt the permeable membrane, denature intracellular proteins, cause DNA damage, and result in membrane leakage. These effects are attributed to the action of Zn2+, which mediates broad-spectrum antibacterial activity against β-lactam-resistant Gram-negative food pathogens through oxidative stress, lipid peroxidation, membrane damage, β-lactamase enzyme inhibition, inactivation of intracellular proteins, DNA damage, and ultimately, cell death (Krishnamoorthy et al., 2022). Furthermore, the toxicity mechanism of ZnO NPs varies depending on the medium due to variations in the species of dissolved Zn (Li et al., 2011). The antimicrobial activity of these nanoparticles has been shown to reduce bacterial burden, skin infections, inflammation, and improve the architecture of infected skin in mouse models (Pati et al., 2014).

# MATERIAL AND METHOD

This section introduces the materials and methods used in the study.

## 3.1. Materials

This section introduces the research materials.

### 3.1.1. Instruments and equipments

Instruments and equipment used in this study and their manufacturer and suppliers are listed in (Table 3.1).

**Table 3.1.** Instruments used in the study with their company and origin.

|  |  |
| --- | --- |
| **Equipment and Instruments** | **Company /Origin** |
| Autoclave | Gallenkamp /England |
| Burner | Amal /Turkey |
| Centrifuge | Eppendorf/ Germany |
| Densichek Plus | Biomerieux/France |
| Disposable Petri dishes | Al-Hani /Lebanon |
| Distillation | GFL/Germany |
| Deep Freeze -80 | Binder / Germany |
| electrophoresis system | cleaver/United Kingdom |
| Gel Documentation | cleaver/United Kingdom |
| Incubator | Binder / Germany |
| Laminar air flow | Gallenkamp/England |
| Refrigerator | TEKA/Spain |
| PCR Thermocycler | ThermoFisher/ USA |
| PCR Workstation | Cleaver / UK |
| Hot plate with Magnetic stirrer | Gallenkamp/ England |
| Ependroff tubes | Sterillin/UK |
| Microtiter plate 96 well | Bio Basic/ Canda |
| Shaker incubator | GFL/ Germany |

The table summarizes the main equipment and instruments used in the study, along with their manufacturers and countries of origin. The diversity of high-quality devices from various international suppliers ensured the reliability and accuracy of the experimental procedures.

### 3.1.2. Chemicals Materials

Chemicals used in this study and their manufacturer and suppliers are listed in (Table 3.2).

**Table 3.2.** Chemicals used in the study with their company and origin.

|  |  |
| --- | --- |
| **Substances** | **Company (Origin)** |
| Absolute ethyl alcohol (99.9%) | Diamond (France) |
| Agar | Himedia (India) |
| Agarose | Cleaver (England) |
| Crystal violet | Himedia(India) |
| Deionized Distillate water | Bioneer (Korea) |
| DNA marker (100-1000)bp | NEB (England) |
| Ethanol 96% | Sigma (USA) |
| Ethidium Bromide | Promega (USA) |
| Free nuclease water | NEB (England) |
| Glycerol | Riedel-Dehaeny (Germany) |
| LL-37 Peptide | Eurogentec (Belgium) |
| Normal saline | Pioneer (Iraq) |
| Red safe dye | Intron (south korea) |
| Resazurin dye | Sigma-Aldrich (Germany) |
| SYBR green | Promega (USA) |
| TAE Buffer (50x) | Carl Roth (Germany) |
| TRIzol Reagent | Thermo Scientific (USA) |

The table lists the key substances used in the study, along with their manufacturers and countries of origin. The selection of high-purity reagents from reputable international suppliers contributed to the accuracy and reproducibility of the experimental results.

### 3.1.3. Culture media

Cultures media used in this study are listed in (Table 3.3).

**Table 3.3.** Culture media used in this study.

|  |  |  |  |
| --- | --- | --- | --- |
| **No.** | **Media** | **Company** | **Origin** |
| 1 | Brain Heart Infusion Agar | Himedia | India |
| 2 | Cetrimide Agar | Himedia | India |
| 3 | MacConkey Agar | Himedia | India |
| 4 | Muller-Hinton Agar | Oxoid | England |
| 5 | Muller-Hinton Broth | Oxoid | England |
| 6 | Nutrient Agar | Himedia | India |
| 7 | Nutrient Broth | Mast | England |
| 8 | Tryptic Soy Broth | Himedia | India |
| 9 | Blood Agar | Himedia | India |

Table 3.3 lists the culture media used in this study, including their manufacturers and countries of origin. The selection of standardized and widely accepted media from reputable suppliers ensured optimal growth conditions for the microorganisms investigated.

### 3.1.4. Antibiotics

Antibiotic discs used in this study are listed in (Table 3.4).

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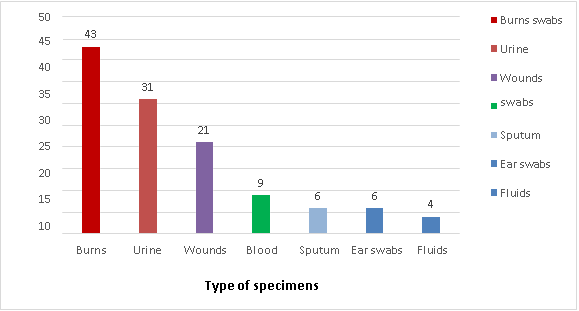
## 3.2. Methods

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# RESULT AND DISCUSSIONS

## 4.1. Description and Distribution of the Study Samples

The current study included the collection of (120) clinical specimens were collected from patients suffering from different infections; (31) urine, (21) burn swabs, (43) wound swabs, (9) blood, (6) sputum, (6) ear swabs and (4) fluids as seen in Figure (4.1). These specimens were collected from patients admitted to four Baghdad hospitals.



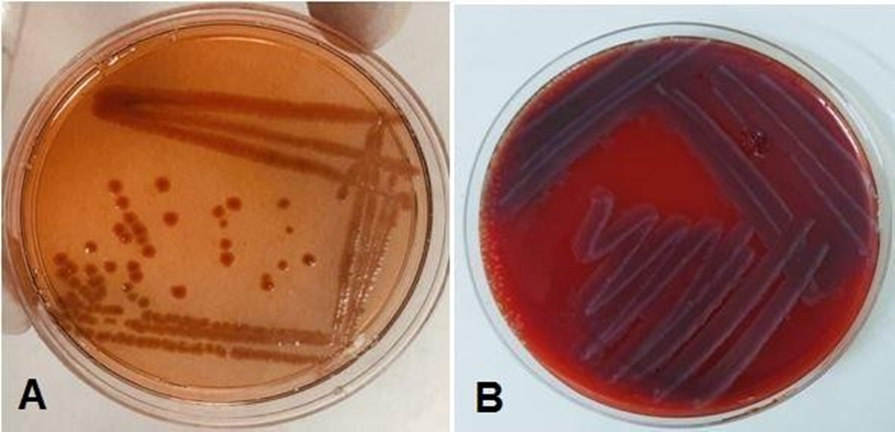
**Figure 4.1.** Number and prevalence of specimens collected from patients in the current study.

## 4.2. Isolation and Identification of *Pseudomonas aeruginosa*

This section describes the methods used for isolating and identifying *Pseudomonas aeruginosa*.

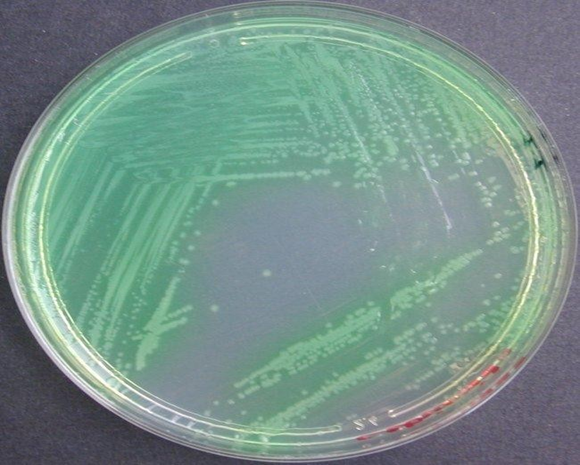
### 4.2.1. Cultural characteristics

All 120 samples were cultured by streaking on Blood, MacConkey, and Cetrimide agar and incubated for 24 hours at 37 °C. The findings showed that the colony on the blood agar is composed of bacteria with sticky textures, a white to a gray hue, and the ability to hemolysis blood, and the type of hemolysis was beta. According to Korgaonkar et al. (2013), *P. aeruginosa* colonies on blood agar frequently display beta hemolysis, a metallic sheen, and can exhibit blue or green pigment. On MacConkey agar, the colonies appear as lactose non-fermenting with small, pale colonies, as depicted in Figure (4.2).



**Figure 4.2.** Colonies of *P. aeruginosa* on (A) macconkey agar and (B) blood agar after incubation at 37˚C for 24 hrs.

The combination of two metabolites produced by P. aeruginosa, pyocyanin (blue) and pyoverdine (green), gives rise to distinctive coloration in cultures, such as the colonies observed on Cetrimide Agar. Cetrimide Agar is a selective and differential medium utilized for the isolation and identification of *P. aeruginosa* from both clinical and non-clinical specimens. Cetrimide acts as a selective agent, inhibiting the growth of most bacteria by functioning as a detergent (specifically, Cetyltrimethylammonium bromide, a quaternary ammonium cationic detergent) (Priyaja et al., 2014). Positive results for *P. aeruginosa* are indicated by colonies displaying a yellow-green to blue coloration, as illustrated in Figure (4.3).



**Figure 4.3.** Colonies of *P. aeruginosa* on Cetrimide Agar after incubation at 37˚C for 24 hrs.

This outcome is comparable to that of the work conducted by AL- Rubaye et al. (2015), who identified Pseudomonas using cetrimide agar and different media. The Pseudomonas spp. Colonies on this medium have a mucoid, smooth shape with flat sides and a raised core that has a fruity scent. The growth of *P. aeruginosa* colonies on Nutrient agar was characterized by the development of pigments and a distinctive grape-like odor, with the colonies exhibiting a greenish coloration, consistent with previous studies (DeBritto et al., 2020). These bacterial colonies displayed Beta-hemolysis (β) on blood agar, indicating the production of hemolysin (Salm et al., 2016). They also produced a blue and green pigment known as pyocyanin, while none of the isolates grown on King B agar showed pyocyanin production. This difference is attributed to the presence of adequate concentrations of potassium and magnesium salts in King A medium, which inhibit the development of fluorescein (Pyoverdin) in King B medium. The production of King B agar is a distinctive feature that differentiates *P. aeruginosa* from *P. fluorescens* (Douraghi et al., 2014).

### 4.2.2. Biochemical Tests

Single greenish colonies were selected from sterile cetrimide agar plates and inoculated into slants for further characterization using various biochemical tests. These tests included the oxidase test, catalase test, nitrate reduction, indole test, methyl red test, Voges-Proskauer test, citrate utilization, and glucose fermentation test, as described by Banerjee et al. (2017). Positive samples were tested twice to ensure accurate results. The confirmation of the identification of all *P. aeruginosa* isolates was performed based on the results obtained from the VITEK 2 System.

### 4.2.3. The frequency of *Pseudomonas aeruginosa*

According to the results of biochemical tests and the VITEK 2 system, of all 120 clinical specimens, 55 *P. aeruginosa* isolates (45.8%) were found in all samples taken from four hospitals in Baghdad. The prevalence of these isolates was 28 (50.9%) in female patients, compared to 27 (49.0%) in male patients, as shown in table (4- 1). The highest rate of bacterial infection was within the age group 31(20-30year), followed by 12 (31-40year), 10(41-50year), and 2(61- 70year), respectively; a significant difference (P<0.001) in the age was observed, as shown in Table (4.1).

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# CONCLUSION AND RECOMMENDATIONS

This section summarizes the key findings of the study and offers recommendations based on the results.

## 5.1. Conclusion

1. The effect of ZnO-np on biofilm formation among strong biofilm producer's strains of *P. aeruginosa* demonstrated an obvious inhibition of the biofilm formation.

2. This study indicated the role of ZnO-NPs as adjuvant with the active antibiotics against *P. aeruginosa* to control infection with this pathogen.

3. It was obvious there was a significant antibiofim effect on the multidrug resistant isolates especially on the strong biofilm producers.

4. The results revealed a significant down-regulation in biofilm genes (lasI, and rhlI) in the presence of sub-MIC doses of ZnO-np.

5. The results showed a significant positive correlation between gene expression of, lasI, and rhlI genes and biofilm formation by using Pearson correlation analysis which included all the tested isolates before and after the treatment with ZnO-np.

## 5.2. Recommendations

1. Study the effect of the combination between nanoparticles and natural products from plants on bacterial biofilm.

2. An *in vivo* experiment complemented this study, especially for the treatment of burn infections.

3. Using other types of nanoparticles for eradication the biofilm formation and studies its effect on the gene expression of related biofilm genes.

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# APPENDİCES

# Appendix 1

# **Conference Participation Certificate** / **First Page of the Article**

# CURRICULUM VITAE

|  |  |
| --- | --- |
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| **Institute** | Institute Of Natural And Applied Sciences |
| **Department** | Department Of Molecular  Biology And Genetics |
| **Graduation Year** | 2023 |

|  |
| --- |
| **Articles and Papers Produced from the Thesis** |
| Malaa, A. S., & Tutar, L. (2023, May). Effect of nanoparticles on the gene expression of virulence factors of *Pseudomonas aeruginosa* [Oral presentation]. *6th International Scientific Research and Innovation Congress*. |