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ANTIBIOTIC RESISTANCE PROFILES OF *PSEUDOMONAS AERUGINOSA* ISOLATES ISOLATED FROM CLINICAL SAMPLES, AND EVALUATION OF BIOFILM FORMATION AND THE PRESENCE OF BIOFILM-RELATED GENES (*pelA*, *pslD* AND *algD*)

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October 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

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ABSTRACT

ANTIBIOTIC RESISTANCE PROFILES OF *PSEUDOMONAS AERUGINOSA* ISOLATES ISOLATED FROM CLINICAL SAMPLES, AND EVALUATION OF BIOFILM FORMATION AND THE PRESENCE OF BIOFILM-RELATED GENES (*pelA*, *pslD* AND *algD*)

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Master of Science in Biology

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Pseudomonas aeruginosa is identified as an opportunistic pathogen since it predominantly creates nosocomial infections in immune compromised people. The available knowledge about *P. aeruginosa* pathogenesis has primarily come from investigating clinical isolates; in this case there were a total of 100 samples obtained from a variety of locations in Baghdad hospitals and sorted into three groups: clinical (40) isolates from burns and (40) specimen from wounds and (30) from sputum, collected from October 2021 to the April 2022. Twenty isolates of *P. aeruginosa* were found of these samples and they cultured by specific and differential media, Using culture conditions, biochemical assays, and the VITEK-2 compact system, bacteria were identified. For all bacterial isolates, the antibiotic sensitivity test is valid according to the minimum inhibitory concentration (MICs). The maximum level of resistance was demonstrated to be against Tigecycline (8), followed by Cefazolin (64), while high sensitive was to Ciprofloxacin (0.25), followed by Levofloxacin (0.5) and Gentamicin (1), cefepime (1), Ceftazidime (2) and Imipenem (2), Amikacin (2) and Piperacillin /Tazobactum (8) respectively according to MICs results of each antibiotics. Biofilm production was detected using the micro titration plate technique, with the majority of strains (50%) producing moderate biofilms, 35% producing strong biofilms, and 15% producing weak biofilms. When it comes to virulence genes, In 20 isolates, three genes were identified, the *algD* gene was found in 17 isolates (85%), *pelA* was found in 17 isolates (85%), and *pslD* was found in 15 isolates (75%). The genes *algD*, *pslD*, and *pelA* were chosen as virulence factors, the genotypic *algD/pslD/pelA* – pattern, which is responsible for the phenotypic pattern of biofilm development. There were statistically significant variations in genotypic pattern predominance across biofilm forming isolates from various sources, Using Chi-square analysis, a very significant relationship between biofilm forming capability and genetic pattern (p value 0.764) was discovered. In this research we wanted to reveal if there was a linkage between the production of biofilms and the expression of biofilm-related genes (*algD*, *pelA*, *pslD*), and analyzing antibiotic resistance profiles in *P. aeruginosa* clinical samples isolated.

2022, 103 pages

Keywords: Biyofilm oluşumu, *Pseudomonas aeruginosa*, *pelA*, Polisakkarit

ÖZET

KLİNİK ÖRNEKLERDEN İZOLE EDİLEN *PSEUDOMONAS AERUGİNOSA* İZOLATLARINDA ANTİBİYOTİK DİRENÇ PROFİLLERİ, BİYOFİLM OLUŞUMU VE BİYOFİLM İLE İLİŞKİLİ GENLERİN (*pelA, pslD* VE *algD*) VARLIĞININ DEĞERLENDİRİLMESİ

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Pseudomonas aeruginosa, bağışıklık sistemi zayıflamış kişilerde ağırlıklı olarak nozokomiyal enfeksiyonlar oluşturduğu için fırsatçı bir patojen olarak tanımlanır. *P. aeruginosa* patogenezi ile ilgili mevcut bilgiler öncelikle klinik izolatların çalışmasından elde edilmiştir. Mevcut çalışmada Bağdat hastanelerinin çeşitli yerlerinden elde edilen ve üç gruba ayrılan toplam 100 örnek vardı: yanıklardan klinik izolatlar (40), yaralardan örnekler (40) ve balgamdan (30). Bu örnekler Ekim 2021'den Nisan 2022'ye kadar toplandı: Bu örneklerden yirmi *P. aeruginosa* izolatı bulunmuş ve spesifik ve farklı ortamlarla kültürlenmiş, kültür koşulları, biyokimyasal analizler ve VİTEK-2 kompakt sistemi kullanılarak bakteriler tanımlanmıştır. Tüm bakteri izolatları için antibiyotik duyarlılık testi minimum inhibitör konsantrasyona (MICs) göre geçerlidir. Maksimum direnç seviyesinin Tigesikline (8) ve ardından Sefazolin (64) karşı olduğu gösterilmiştir. Yüksek duyarlılık ise Siprofloksasin (0.25), sonra sırasıyla Levofloksasin (0.5) ve Gentamisin (1), sefepim (1), Ceftazidime (2), İmipenem (2), Amikasin (2) ve Piperasilin/Tazobactum'da (8) izlenmiştir. Mikro titrasyon plakası tekniği kullanılarak biyofilm oluşumu tespit edildi, suşların çoğunluğu (%50) orta derecede biyofilmler üretti, %35'i güçlü biyofilmler üretti ve %15'i zayıf biyofilmler üretti. Virülans genleri anlamında 20 izolatta üç gen saptandı. 17 izolatta (%85) *algD* geni, 17 izolatta (%85) *pelA* ve 15 izolatta (%75) *pslD* saptandı. *algD, pslD* ve *pelA* genleri virülans faktörleri olarak seçildi, ki bu biyofilm gelişiminin fenotipik paterninden sorumlu olan genotipik *algD* / *pslD* / *pelA* paternidir. Çeşitli kaynaklardan biyofilm oluşturan izolatlar arasında genotipik örüntü baskınlığında istatistiksel olarak anlamlı farklılıklar vardı. Ki-kare analizi kullanılarak biyofilm oluşturma kabiliyeti ile genetik örüntü arasında çok anlamlı bir ilişki bulundu (p değeri 0.764). Bu çalışmada biyofilm üretimi ile biyofilm ile ilişkili genlerin (*algD*, *pelA*, *pslD*) ekspresyonu arasındaki ilişkiyi araştırdık ve izole edilen *P. aeruginosa* klinik örneklerinde antibiyotik direnç profillerinin analizini yaptık.

2022, 103 sayfa

Anahtar Kelimeler: Biyofilm oluşumu, *Pseudomonas aeruginosa*, *pelA*, Polisakkarit

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Noor Riyadh Hamoodah ALMZIL
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LIST OF SYMBOLS

%	Percentage
µL	Microliter
℃	Celsius centigrade
cm	Centimeter
mL	Milliliter
µm	Micrometer

LIST OF ABBREVIATIONS

AME	Aminglycoside modifying enzymes
AST	Antibiotic susceptibility test
BHL	Brain heart broth media
CDS	Centers for disease control and prevention
CF	Cystic fibrosis
CFU	Colony forming units
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
EPS	Extracellular polymeric substances
ETA	Exotoxin A
LPS	Lippopolysaccharide
MDR	Multi drug resistance
MICs	Minimum inhipitory cocentrations
OD	Optical density
PCR	Polymerase chain reaction
PEL	Pelicle formation locus
PSL	Polysacharide synthesis locus
QS	Quorum sensing
RNA	Ribonucleic acid
UTI	Urinary truct infection

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1 INTRODUCTION

Pseudomonas aeruginosa a gram negative bacteria and is the most commonly connected Pseudomonas strain with human illnesses (Streeter and Katouli 2016).

Those are responsible for cystic fibrosis patients' persistent lung infections, in particular ventilator-associated pneumonia, wound infections in severe burn patients, septicemia, and catheter-associated (UTLs), multi kinds of infectious diseases have been identified linked to *P. aeruginosa*, all of which have complicated etiologies (Streeter and Katouli 2016).

As a result, *Pseudomonas aeruginosa* is a widespread nosocomial pathogenic bacteria in hospitals and burn clinics, where it is a leading cause of death (Yang *et al.* 2018).

Planktonic *P. aeruginosa* cells attach to surfaces and adopt a sessile lifestyle in response to environmental stimuli. Sessile cells multiply on surfaces, eventually developing into biofilms, which are encased in a self-made matrix made up of several macromolecules, including exopolysaccharides (EPS) (Yang *et al.* 2018).

The development of a polymeric extracellular matrix is due to Gram-negative bacillus bacteria (which holds the cells of the biofilm community together), and adhesion cells aggregation, and all mechanisms that explain how Gram-negative biofilms form.

In Gram negative bacteria biofilms, polysaccharides (a simple sugar molecule) which serve as a key role in antimicrobial resistance and in the formation of mature biofilms. (Laverty *et al.* 2014).

Virulence factors are particular to mature biofilms production and antibiotic resistance including: (flagellum, type IV pili, extracellular virulence factors, siderophores, hydrogen cyanide, pyocyanin, lipopolysaccharide (LPS) O-antigen, and Quorum

Sensing Systems (QS), lipase, elastases, exotoxin A [ETA], protease IV, exoenzymes S, T and Y, rhamnolipids, exotoxin U, phospholipase C, alginate) (Wu *et al.* 2015).

Antimicrobial resistance obtained by chromosomally encoded systems' expression and/or functions are modified by mutational events, or by acquiring resistance genes on plasmids (Kadum *et al.* 2019).

This bacterium was recognized for producing pigments like (pyocyanin), which were poisonous to a variety of bacteria and fungi, as well as damaging mammalian cells.

Pseudomonas aeruginosa may produce a wide range of exopolysaccharides, including: ((Pel) or pellicle formation locus), (polysaccharide synthesis locus or (Psl)), and alginate, Exopolysaccharides and extracellular DNA are significant elements that aid in the formation of huge aggregates or microcolonies, which are hallmark of biofilms (Kadum *et al.* 2019).

((Pel) or pellicle formation locus) include the *pelA* gene that responsible for biofilm formation. Pel serves as a major structural frame for the population, ensuring cell-to-cell connections in the *P. aeruginosa* biofilm. It also has a secondary effect of increasing antibiotic resistance to aminoglycosides, albeit only in biofilm populations.(Al *et al.* 2019).

(Polysaccharide synthesis locus or (Psl)) contain the *pslD* which is a type of protein secretion involved in the exopolysaccharide export process, which makes it necessary for biofilm development. (Kamali *et al.* 2020).

Alginate has *algD* enzyme that is responsible for converting (GDP manose) into (GDP mannuronic acid) (Kamali *et al.* 2020).

The main gene that stimulates alginate synthesis is *algD*, which is followed by the joint action of *mucA* and *algU* (Pulcrano *et al.* 2012).

According to that, in the current study we aimed to:

- The Determination of Antibiotic Resistance in *Pseudomonas aeruginosa*: (β-lactam, fluoroquinolones, cephalosporins, glycylcycline, carbapenems and aminoglycosides antibiotics), to investigate if there's a class of synthetic wide-spectrum antibiotics that kill bacteria by inhibiting cell wall, cell membrane, protein synthesis or DNA synthesis.
- Investigate the frequency of the Genes carried by *P. aeruginosa*, *pelA*, *pslD* and *algD*, and how they are associated with pathogenicity above the average of other bacterial infections in hospitals. In addition evaluation the apparent correlation of polysaccharides genes among biofilm producing *P. aeruginosa* clinical isolates.

2 LITERATURE REVIEW

2.1 *Pseudomonas aeruginosa*

It was found that *P. aeruginosa* through exposed to ultra-violet light, the blue-green pigments water-soluble appears. It was made by the scientist French bacteriologist and chemist (Carle Gessard) (D’Andrea *et al.* 2018)

Pseudomonas aeruginosa is highly aerobic encapsulated and a gram-negative, has a length of 1.5 to 3 µm and a width of 0.5 to 0.7 µm rod-shaped straight or slightly curved (it can grow anaerobically in the presence of nitrates), Plants and animals, including humans, are susceptible to this pathogen. (Diggle and Whiteley 2020)

Because of *P. aeruginosa's* widespread lifestyle, this bacterium contributes to frequent infections in humans. It is a very versatile bacterium, with soil being its preferred home; nevertheless, *P. aeruginosa* can also thrive in watery settings. *P. aeruginosa* can withstand the breakdown of hazardous waste due to its nutritional variety (Fujitani *et al.* 2017).

It is also a member of the normal flora by its anatomic location in skin and colon, which are low-virulence organisms in the body and they are nonpathogenic in these location, in immune compromised people particularly if they leave their regular anatomic place, they might cause sickness. (Levinson 2020).

Because of its natural resistance to medicines, treating *P. aeruginosa* infections can be difficult. Adverse effects may arise when more strong antibiotic treatment regimens are needed, the organism is opportunistic and is known as severe contamination frequently happens in the course of current illnesses or conditions – maximum appreciably cystic fibrosis and traumatic burns (Lamas Ferreiro *et al.* 2017).

P. aeruginosa generally only wants acetate and ammonia for nutrition because of the supply of carbon and nitrogen, respectively *P. aeruginosa* grows anaerobically, it does not ferment, but gains energy from the oxidation of sugars, the diverse nutrient requirements enable it to grow in peripheral areas.

Growth of *P. aeruginosa* on amino acids or citrate favors the creation of flat, homogenous biofilms, whereas growth on glucose promotes the formation of structured biofilms with large cell aggregates (Perez *et al.* 2011)

P. aeruginosa isolates produce 3 sorts of colonies.

- Rough and small colony which represent natural isolates taken from soil or water usually.
- At the same time as clinical isolates are probably smooth colony types, with big fried-egg appearance, smooth, with flat edges and an increased appearance.
- Mucoid type consistent with the existing of (alginate slime which is an exopolysacchride consisting of gulcuronic and mannuronic acids) in respiratory and urinary tract secretions.

Pyoverdin and pyochelin are fluorescent siderophores produced by the bacterium, which has the ability to scavenge iron, and pyocyanin, an oxidant pigment that gives *P. aeruginosa* its blue color (Long *et al.* 2017)

2.2 Taxonomy of *P. Aeruginosa*

- Kingdom: Bacteria
- Phylum: Proteobacteria
- Class: Gammaproteobacteria
- Order: Pseudomonadales
- Family: Pseudomonadaceae
- Genus: Pseudomonas

- Species: Pseudomonas aeruginosa (Cavalier-Smith 2004)

Many species produce siderophores as secondary metabolites to scavenge iron from their environment, allowing the cell to acquire this critical element. Siderophores make soluble ferric complexes that organisms may consume due to their high affinity for ferric iron. (Albelda-Berenguer *et al.* 2019)

2.3 General Characteristics

Pseudomonas aeruginosa a Gram-negative and a typical encased, non-spore, rod-shaped of 0.5–0.8 µm by 1.5–3.0 µm, a sporogenous, Mono flagellated bacteria can be found as singles, pairs, or short chains. It has a pearlescent look and smells like grapes or tortillas. (Wu *et al.* 2015).

It may be found in soil, water, plants, and even people. It's found in large amounts on fresh fruits and vegetables. *P. aeruginosa* may grow at temperatures as high as 42°C, while 37°C is the optimal temperature for growth, which sets it apart from other Pseudomonas species (Prince 2012).

Pseudomonas aeruginosa produces soluble pigments such pyocyanin (non-fluorescent blue), the fluorescent pyoveridin (yellow-green), pyomelanin (brown), and pyorubin (red). (Behzadi *et al.* 2021).

P. aeruginosa makes pyocyanin, that is basically a phenazine with blue-green pigment (N-methyl-1-hydroxyphenazine), occur in vitro as water soluble and generated in huge amounts, also It is citrate, catalase, and oxidase positive (Blankenfeldt and Parsons 2014).

P. aeruginosa has a single circular chromosome that makes up its genome. the genome has high content of G + C (65-67%) and rather big (5.5 - 7 Mb), and is known to harbor plasmids that are easily interchangeable (Tang and Sails 2014).

The genome of this bacterium, which encodes regulatory genes that cover around 8% of the organism, allows it to adapt to complicated growth settings.

It is classified opportunistic pathogen in nosocomial infections related to high nutritional versatility and resistance to various antimicrobial agents, *P. aeruginosa* encodes a huge number of extracellular enzymes due to its vast genome. And an extensive slime layer for various metabolic pathways (Tang and Sails 2014).

To develop an infection, *P. aeruginosa* takes advantage of deficiencies in the host's defenses; the symptom (blue pus) is indicative of Pyocyanin, a common feature of P. aeruginosa infections (Wu and Li 2015).

P. aeruginosa environmental reservoirs in hospitals include sprays, drinking water, taps, sink and bath drains, breathing apparatus, air purifiers, bath basins, lens cleaners, therapeutic or swimming pools, In addition, dialysis tubing, respiratory devices, ventilator buttons, bedrails, and monitors were regularly infected (Bédard *et al.* 2016).

Pseudomonas aeruginosa can spread by patient-to-patient transmission and environmental contamination, among other methods. In a hospital setting, it may live on inert, dry surfaces for 6 hours to 6 months due to its adaptive nature and great survival capacity.

Another source of infection dissemination that should not be overlooked is operator hands, After coming into touch with infected patient or using polluted soap, lotion, or water, which can become contaminated. (Pachori *et al.* 2019).

2.4 Identification

P. aeruginosa was isolated using multiple selective media which used to be incubated at 37^{o}C for 24-48 hours, including MacConkey agar, Muller Hinton agar, and blood agar.

In order to identify which isolates are present, routine tests for instance pigment biosynthesis on selective medium and colony morphology, glucose fermentation, gelatin hydrolysis, positive oxidase test, and the right temperature used for the growth were at 42ºC. (David *et al.* 2020)

Bacterium's biological properties under certain culture conditions controlled the traditional *Pseudomonas aeruginosa* detection methods, for example (Gram-positive and negative status), as well as the capabilities of microorganism enzymes for instance, essential amino acid dihydrolase, pyocyanin and oxidase (Gagliardo and Saiman 2015).

McConkey agar, which is based on lactose fermentation, is a selective and differential culture medium.(Colorless colony) with green pigmentation, Does not ferment lactose.

On Cetrimide agar (a selective media derived from clinical samples) which increases pyoverdine and pyocyanin synthesis, while inhibits other bacteria.

Large, low, oval, convex, rough, and occasionally surrounded by serrated growth on nutritional agar for 24 hours at 37°C. (David *et al.* 2020)

It generates huge flat spreading, hemolytic, tough, mucoid, pigmented colonies with a distinct metallic sheen on blood agar.

The presence of indophenol oxidase, citrate, and l-arginine dehydrolase activities in *P. aeruginos*a may be determined biochemically (Streeter and Katouli 2016).

It is positive for citrate, catalase and oxidase, methyl red/Voges Proskauer test negative, does not form phenyl pyruvic acid, mannitol fermented, indole negative, does not hydrolyze urea and liquefies gelatin. Neither glucose (red broth) nor hydrogen sulfide are fermented by this organism (H_2S). (Moehario *et al.* 2019).

2.5 Epidemiology

In patients with nosocomial pneumonia and burn wound infections, *P. aeruginosa* is the most usually observed pathogen, (11–13.8%) of all nosocomial pathogens transmitted by bacteria following microbiological isolation.

P. aeruginosa can spread through a variety of pathways, including patient-to-patient transmission and environmental contamination.

Due of its adaptation and high survival ability, it can live for 6 hours to 6 months on dry abiotic surfaces in a healthcare setting. (Pachori *et al.* 2019).

Because *P. aeruginosa* may be found practically anyplace, it is nearly hard to avoid exposure. Insufficient cleaning or disinfection of reusable medical instruments after procedures including, endoscopy, retrograde cholangiopancreatography (ERCP), ophtalmic equipment, and transrectal ultrasonography has been linked to clusters of *P. aeruginosa* bacteremia/sepsis (TRUS) (Fujitani *et al.* 2017).

Due to the strong preference of the organism for water, it’s been found in cosmetics, hot tubs, soaps, contact lens solutions and disinfectants, all of which have been recognized as possible infection causes. It is frequently seen in small quantities in the gastrointestinal tract, although it is not a common component of human natural flora. It can colonize damp skin regions like the underarm and the perineum. *P. aeruginosa* has been shown to invade the throat and nose as well (Moore and Flaws 2011).

Acute and persistent lung infections, neonatal sepsis, and burn sepsis have all been associated to this bacterium. It's a microbe that infects people with AIDS and diabetes. In addition, cystic fibrosis patients are more likely to develop chronic pulmonary disease. Hematologic malignancies, human immunodeficiency virus (HIV) or (AIDS), and diabetes mellitus are only a few examples of chronic neutropenia and neutrophil function abnormalities. In fact, chronic pulmonary illness is prevalent in cystic fibrosis patients.

It may be found in living things and because of its non-fastidious character and low nutritional requirements; it is seen in hospital sinks, toilets, showers, and patient care equipment, particularly respiratory ventilators. New vegetables and fruits have also been shown to have *Pseudomonas aeruginosa* on their surfaces (Fujitani *et al.* 2017).

Pseudomonas aeruginosa can infect nearly any region of the body, although it rarely infects a healthy host. People who have damaged mucous membranes or skin which no more function like infection-controlling barrier are the most vulnerable to *P. aeruginosa* infections (e.g. in burn patients).

The fourth most common illness is urinary tract infections (UTIs) which is coming from *P. aeruginosa* infection, as well as it is the second most prevalent reason of respiratory infections in Surgical patients, recently reported by (CDC) Centers for Disease Control and Prevention, while the (blood stream infection) (BSIs) are the sixth most common cause (Moore and Flaws 2011).

This bacterium is a common opportunistic that infects persons with weakened host defenses, such as:

2.5.1 Chronic and acute burn wounds

Soft tissue infections are among the most serious of these diseases, including chronic and burn wounds, *P. aeruginosa* burn infections can spread fast, resulting in systemic dissemination and mortality within days or weeks, However, normal *P. aeruginosa* infections occur many weeks after the original burn (Oumeri and Yassin 2021).

The most prevalent cause of serious burn wound infection is significant immunosuppression. Burns affect both the general and specific components of the immune system (Gonzalez *et al.* 2016)

After the fifth day, bacterial growth begins at the deep surface of the burn wound's eschar. Eschar, as a dead tissue, functions as a culture medium and promotes bacterial growth. This bacterial growth in the sub Escher may eventually infiltrate deeper tissues and cause burn wound sepsis (Cartotto 2017).

2.5.2 Cystic fibrosis

The greatest reason of morbidity and death and also called the chronic airway infection, the special lung environment that incident in cystic fibrosis patients promotes a persistent disease with *P. aeruginosa*, that exhibits a distinctive mucoid morphology due to the formation of alginate which involves the micro colonies of the organism's (Maurice *et al.* 2018).

In *Pseudomonas aeruginosa* specimens from CF patients' sputum, a mucoid exopolysaccharide coating was recently found (Maurice *et al.* 2018).

2.5.3 Pneumonia or bacteremia

P. aeruginosa pneumonia or bacteremia is common in hospitalized patients with malignancy, diabetes, or heart disease, especially those on medical respirators. 1-3 *P. aeruginosa* infections in hospitalized patients result with a longer length of stay (LOS), higher expenditures, with a high death and morbidity rate.

Exopolysaccharide, which permits bacteria to develop micro colonies in the lungs, is a crucial component in *P. aeruginosa* pneumonia (Maurice *et al.* 2018).

2.5.4 Community-acquired pneumonia (CAP)

In hospitalized patients, the bacterium *Pseudomonas aeruginosa* is a common nosocomial infection that causes pneumonia. The majority of these individuals has a pre-existing medical condition or is at risk for Pseudomonas infection. Although rare, there have been cases of previously healthy people developing *P. aeruginosa*-caused community-acquired pneumonia (CAP).

It is a condition in which a person who has not been hospitalized develops an acute infection of the lungs (Fujii *et al.* 2014)

2.5.5 Pneumonia associated with a ventilator (VAP)

Among patients, it is the most prevalent infection, as well as the most common reason for antibiotic prescriptions in intensive care units (ICUs), with high death rates (about 13%), extended hospital stays, and rising healthcare expenditures (Aykac *et al.* 2017)

P. aeruginosa causes (VAP) by bacterial spread/aspiration to the lower respiratory tract. Infection is more prevalent in those who have chronic illness like hypo-gamma globulinemia or neutropenia also who used respiratory support infected by cystic fibrosis, or are immune compromised such as cancer (Kalil *et al.* 2016).

2.5.6 Endophthalmitis

P. aeruginosa endophthalmitis is a medical problem caused by a bacterial invasion of the vitreous and/or watery humors within the eye. Exogenous cases are the most common, occurring after eye surgery, severe ocular trauma, or a corneal infection (Durand 2013).

2.5.7 Infections of the skin and soft tissues

2.5.7.1 Folliculitis

Folliculitis (pyogenic or pustular) is a skin infection that is frequently associated with the usage of a swimming pool or a hot tub. About 67% of hot tubs the bacterium *Pseudomonas aeruginosa* has been discovered and also in 63% of swimming pools (Jacob and Tschen 2020).

2.5.7.2 Nail diseases

Patients who are susceptible to *P. aeruginosa* infection (for example, onycholysis) are commonly referred to as having "green nail syndrome (Ohn *et al.* 2020)

2.5.7.3 Foot infections and necrotizing fasciitis

The moist inter digital regions of the feet, especially diabetic foot infections, are excellent locations for *P. aeruginosa* colonization (Alkhudhairy and Al-Shammari 2020). The germs can migrate to the skin, causing ecthyma gangrenosum, which are black, necrotic sores (Levinson 2020).

2.5.8 Neutropenia

Despite the fact that neutropenia is still a significant risk factor for severe *P. aeruginosa* infections. When *P. aeruginosa* infection arises, mortality might reach 50-70% (Hansen *et al.* 2020).

2.5.9 Human immunodeficiency virus HIV/AIDS

Infections with *P. aeruginosa* have been recognized as nosocomial and community-acquired in adult and children. HIV/AIDS occurs in those who suffering from low CD4 cell levels. A prior diagnosis of AIDS was strongly associated with respiratory illness, which accounted for 47% of all cases (Rooku *et al.* 2019).

2.5.10 Otitis media

Chronic supportive otitis media (CSOM) is a common and well-known illness in the middle ear in impoverished nations, causing significant local damage as well as life-threatening consequences (Sahu *et al.* 2019).

Malignant otitis externa: sever infection of the external ear and skull base that needs prompt diagnosis and treatment. It affects those who have a weakened immune system, notably diabetics (Arsovic *et al.* 2020)

2.5.11 Urinary tract infections (UTI)

The most frequent nosocomial infections, the rate reaches approximately 20 to 49% *P. aeruginosa* UTI infection can develop in people who have a neurogenic bladder (Lamas Ferreiro *et al.* 2017).

2.5.12 Pathogenesis

In the absence of compromised host defenses, *P. aeruginosa* does not induce infection. Several possible virulence factors have been found that may contribute to its pathogenicity when a patient's immune system is depleted (Maurice *et al.* 2018).

There are a lot of regulatory genes that are involved in environmental sensing, virulence factor expression regulation, metabolism, and resistance mechanisms as a result of its huge genome (>6 Mb) which encodes all of these mention above (Faure *et al.* 2018).

Another example is the secreted virulence factors: phospholipase C (heat-labile hemolysin) In vivo, strong toxins can cause bleeding, edema, and tissue necrosis by damaging cells, including red blood cells (Ms *et al.* 2021).

These germs can multiply in settings that would not ordinarily support high bacterial growth, such as salt solutions, soaps, and mild antiseptics, because they get their carbon and energy from basic organic molecules.

Other bacteria synthesize all of the virulence components needed by *P. aeruginosa*, but only *P. aeruginosa* produces pyocyanin.

This organism located in the environment and as (normal flora) in microbiota of the gut as well as in the skin (Spagnolo *et al.* 2016).

It is a characteristic bacterium of biofilms; it can readily attach to moist or liquid-contact surfaces.

Aerosols, drinkable water, Bathing basins, taps, sink and shower drains, respiratory devices, air purifiers, endoscope cleaners, water baths and therapeutic or swimming pools have all been identified as confirmed *P. aeruginosa* environmental reservoirs in a hospital context (Bédard *et al.* 2016).

All of these processes cause local inflammation and tissue injury, allowing *P. aeruginosa* to penetrate the host and get nutrients. *P. aeruginosa* antimicrobial resistance is a health issue that can lead to treatment failure, besides the biofilm formation phase defends against antibiotics (Fujitani *et al.* 2017).

P. aeruginosa is considered an extremely dangerous infection as reported in the World Health Organization (WHO). because of its potential to cause bloodstream infection (septicemia) and drug resistance (Spagnolo *et al.* 2016).

2.6 Virulence Factor

P.aeruginosa has virulence factor that help it colonizes the organism and manage to avoid the immune system in order to infect the organism, including: (Behzadi *et al.* 2021)

They are classified into two classes based on their function: those that help in organism adhesion to host cells, those that help in tissue penetration and immune response suppression, which including fimbriae as well as flagella, With the exception of pyocyanin, which is generated only by *P. aeruginosa* (Moore and Flaws 2011).

Virulence factors are classified into two parts:

Acute affect infection: by factors that generated or found on the bacteria surface.

- Metabolites (phenazine/pyocyanin)
- Pili that allow for epithelial adhesion
- Exoenzyme S enhances epithelial cell adhesion; the pathogenicity of exoenzyme S is linked to cytoskeletal structural disturbance. Actin filament depolymerization occurs when immunoglobulin G and A are broken down, adding to macrophage resistance.
- Exotoxin A: exotoxin A is a toxin that is produced by the body
- Phospholipase C is a thermolabile haemolysin that causes tissue necrosis. (Moissenet and Khedher 2011)

Chronic infection:

- Siderophores comprise the (pyoverdin and pyochelin pigments), which permit bacteria to thrive when ferrous ions is lack, are implicated in chronic infection.
- Alginate, which was found in cystic fibrosis patient strains, contains a pseudo capsule that protects bacteria against Antibiotics, phagocytosis, and dehydration

It also enhances epithelial cell adhesion, enabling biofilm formation (Moissenet and Khedher 2011)

Bacterial systems and variables that are involved in the production of this bacterium biofilms. (1) Exopolysaccharides synthesis and c-di-GMP pool control; (2) (GacS/GacA and RetS/LadS) the two regulatory system components (3) Quorum sensing system (Sarkis-Ivanova 2017).

2.6.1 QS quorum sensing system

QS Is a communications between cells method based on cell density (Cell-to-cell interaction in bacteria) which permit bacteria to manage gene expression via signal molecules called Autoinducers based on the density of the cell population. QS Controls bacteria pathogenicity and biofilm development. The *P. aeruginosa* QS network, which is organized in a multilevel hierarchy with at least four connected signaling systems (Lee and Zhang 2015).

The quorum sensing system has bacteria producing autoinducers, which are small diffusible signaling molecules. Species-specific autoinducers (AI-1) and interspecies communication autoinducers (AI-2) are the two categories of autoinducers (AI) (AI-2) (Saipriya *et al.* 2020).

QS Is an intercellular communication phenomenon in which bacteria may detect and respond to their own cell population and environmental cues by changing genes that help them survive and colonize both biotic and abiotic surface.

P. aeruginosa contains four primary QS systems, it regulates virulence factor gene expression as well as biofilm growth and maturity, including (las, rhl, (PQS) Pseudomonas quinolone signal and (Iqs) (Fila *et al.* 2018).

Focusing on the importance of this novel QS system in bacterial disease control Importantly, IQS found to be capable of taking over parts of the central las system's roles in the presence of phosphate depletion stress (Lin and Cheng 2019)

Peptide synthase, which is not present on ribosomes, is formed by the IQS of gene aggregation ambBCDE. If disturbed, it suppressed the synthesis of PQS and signals of BHL, as well as rhamnolipids, elastase and pyocyanin which are virulence factors (Lee *et al.* 2013).

PQS is mainly composed of HHQ, which may be transferred from one *P. aeruginosa* cell to the next. PqsH a presumed flavin-following mono oxygen seat by introduce a hydroxyl group into HHQ at the 3-position, which transforms HHQ into PQS, The Las system controls the PQS system, which promotes pqsH transcription (Lin and Cheng 2019).

The las system also regulates the third system, PQS, This system is involved in the production of lasB and regulates genes that make elastase, proteases, and pyocyanin. PQS can't work without RhlR (Lee and Zhang 2015).

There are two polypeptide domains in the R protein: a target binding domain (C-terminal) and autoinducer binding domain (N-terminal). The Las-Box, which is 40 kb before the ATG start codon, binds in a helix-turn-helix sequence at the C-terminal region. Las-Box is a palindrome with 20 base pairings, and The C-terminal domain's amino acids aid polymerization (Moghaddam *et al.* 2014).

The C-terminal domain is inhibited from target gene binding by the N-terminal domain when there is no autoinducer available. The Las system has The R protein that binds to the C12-HSL autoinducer, forming a multimer that can attach to upstream of the gene encoding to the Las-Box (Moghaddam *et al.* 2014).

LasR protein/C12-HSL regulates Rh1R gene expression and plays a potent role in the LasI gene regulation, whereas RhlI gene transcription promotes by RhlR protein/C4-HSL (Moghaddam *et al.* 2014).

Bacterial quorum sensing pathways are made up of several important components, such as signal molecules, bacterial populations, target genes and protein activators (Lin and Cheng 2019).

Quorum sensing regulates a wide range of important bacterial function, that comprise, antibiotic biosynthesis, bioluminescence, swarming, swimming, twitching, formation of biofilm and dissemination, conjugation, sporulation, and the production of virulence markers, among others (Saipriya *et al.* 2020).

2.6.2 Biofilm production and its matrices

The initiation of adhesions and extracellular polymeric substances (matrix components) (EPS) function as a framework and encase bacteria in biofilms. is required for bacteria to change from planktonic to biofilm development mode (Ciofu and Tolker-Nielsen 2019).

The composition of matrix of these bacteria biofilms is strain-dependent, in addition growth circumstances and biofilm age (Ciofu and Tolker-Nielsen 2019)

The majority of EPS is made up of polysaccharides (lipopolysaccharide, alginate), extracellular DNA (eDNA), proteins (elastace, protease), minerals, metabolites (pyocyanin, phenazines), siderphorse and exotoxin.

Biofilm growth is a protective mode of reproduction which enables bacteria to survive under difficult conditions. Also allowing seeded cells to invade new niches with optimal conditions (Yin *et al.* 2019).

It's a type of bacterial activity occurs by the surface and controlled by sophisticated signaling networks named quorum sensing (QS) (Olivares *et al.* 2020).

Biofilms are able to form on a variety of surfaces and in a variety of environments, including natural, industrial, and medicinal ones. Sedimentary and planktonic microbial communities differ physiologically (Yin *et al.* 2019).

Biofilm production is a never-ending cycle in which structured colonies of bacteria are bound to surface by the (EPS extracellular polymeric substances matrix).

Microbial cells are held together by EPS, which acts as a matrix or glue. Biofilms' overall architecture and resistance characteristics are influenced by the biofilm matrix (Yin *et al.* 2019).

The glucose-rich polysaccharide is an essential element of biofilm because it is required for biofilm to form on a surface.

Polysaccharides, extracellular DNA, siderophores, proteins, RNA and metabolites such as pyocyanin are all self-synthesised macromolecules in *P. aeruginosa* biofilms (Spagnolo *et al.* 2016)

Proteinaceous membrane attachments, that seems to be due to their nature involved in biofilm matrix components for instance flagellum, (T4P) fimbriae type IV pili, eDNA, Proteins, Exopolysaccharides will be described below:

2.6.2.1 Exopolysaccharide (Psl, Pel, and alginate)

Alginate, Psl, and Pel are three extracellular polysaccharides (or exopolysaccharides) produced by PA. They have a variety of protective characteristics, as well as surface and self-adhesion properties. They are biofilm matrix components that have a role in surface colonization, antibiotic resistance and host immune evasion (Jones and Wozniak 2017).

- **Psl (polysaccharide synthesis locus)**

The Psl cluster of the PAO1 polysaccharide synthesis locus in *P. aeruginosa* comprice of (15 fifteen co-transcribed (*pslA* to *pslO*) genes which encode proteins that produce Psl, This is required for the formation and constant biofilms, as well as contributing to biofilm antibiotic tolerance through facilitating cell-to-surface and cell-to-cell communication (Tariq *et al.* 2020).

Psl is a neutral polysaccharide, the l-rhamnose, d-mannose, d-glucose, all of them are component in Psl that has been discovered and named as a repeating pentasaccharide.

Psl was identified to be an exopolysaccharide rich in galactose and mannose with low quantities of glucose and xylose (Tariq *et al.* 2020).

Exopolysaccharide production and biofilm development are both regulated by the psl operon.

pslD is a type of protein secretion involved in the exopolysaccharide export process, which makes it necessary for biofilm development.

The Psl polysaccharide triggered the cell-surface and intercellular adhesion in *P. aeruginosa*. indicating that It is necessary for the production of biofilms and maintenance (Kamali *et al.* 2020).

Psl polysaccharide on the surface of the cell membrane has a helical structure that allows for strong bacterial cell-cell contacts. During the biofilm development initial stages, this leads in the formation of a biofilm and its matrix. During biofilm development, Psl polysaccharide collected along the edges of three-dimensionally organized macro colonies. The structure was supported by this localization pattern, which allowed for subsequent biofilm dispersal (Al-Wrafy *et al.* 2017).

Psl drive to biofilm development through acting as a route for events as chemical signal as well as a scaffolding for biofilm. *P. aeruginosa* has an evolutionary advantage in colonizing diverse habitats because of this positive feedback circuit. Psl also serves as a defense against immunological and antibiotic assaults (Billings *et al.* 2013).

- **Pel Polysaccharide (pellicle Formation)**

Pel polysaccharide is an extracellular matrix with glucose-rich and cellulose-sensitive synthesis by the pel gene cluster's derivatives.

In standing cultures, pellicle formation at the air-liquid interface appears to be inhibited in a pel mutant. Pel is also needed for formation of biofilm on solid surfaces. (Kamali *et al.* 2020).

Pel production necessitates the products of the *pelA-pelG* gene operon, all of them necessary for Pel-linked behaviors and biofilm development. *pelA*, for example, encodes a polysaccharide deacetylase domain which is a protein that has been predicted. In the opportunistic pathogen *Pseudomonas aeruginosa*, Pel polysaccharide functions as an intercellular adhesion molecule, allowing biofilm development and stability (Colvin *et al.* 2013)

Trans membrane proteins include *pelD* and *pelG*, *pelA* is an oligo galacturonide lyase, both (*pelC*, *pelf*) are glycosyl transferases, synthase of sucrose (*pelE*), Biofilm production was significantly hampered by the addition of transposons into or removal of these genes. There are seven genes in the pel operon with sequences that are similar to sugar-processing enzyme genes from other organisms (*pelA* through *pelG*) (Ghafoor *et al.* 2013).

In PA14 biofilms, the Pel polysaccharide act as a structural framework potentially to the cell population, preserving cell-to-cell interactions and enhancing biofilm resistance to aminoglycoside antibiotics (Al-Wrafy *et al.* 2017).

Friedman and Kolter claimed that five of the seven pel gene comprise (*pelA*, *pelC, pelD, pelE, and pelF*), products had genetic similarity with specific proteins in carbohydrate synthesis (Kamali *et al.* 2020)

Pel genes code for proteins that resemble polysaccharide biosynthesis components (Marmont *et al.* 2017)

Each genes identified in this manner: *pelG* is a 12-integral membrane protein while *pelA* is an oligogalacturonide lyase found in the cytosol; an outer cell envelope protein is the (*pelB*); periplasmic glycosyltransferase include(*pelF* and *pelC*); *pelD* and *pelE* are both large cytosolic proteins acting as an cytoplasmic cell membrane integral protein (Ghafoor *et al.* 2013).

The first gene *pelA* in the operon is essential for Pel biosynthesis and for creating biofilm structure (Szymańska *et al.* 2020).

pelA may also work as a hydrolase, which is essential for assessing the size of Pel polymers and/or removing polysaccharide from the periplasm (Marmont *et al.* 2017).

- **Alginate**

Pseudomonas aeruginosa biofilms comprise the exopolysaccharide alginate, which is an essential element (Tan *et al.* 2014).

Alginate is a polymer which is linear and non-branched and made up of D-mannuronic acid and L-guluronic acid (Pachori *et al.* 2019).

The algACD operon controls the formation of alginic acid, often known as alginate, in *Pseudomonas aeruginosa*. Multiple environmental variables influence the up regulation of alginate-related genes, including high oxygen concentration, high osmolality, nitrogen deficiency, and the presence of ethanol. (Moradali *et al.* 2017)

It discovered that some enzymes required to encode and create the alginate precursor (GDP) guanosine diphosphate mannuronic acid include (*algA*, *algC*, and *algD*) and because of that Alginic acid production requires these enzymes.

GDP manose dehydrogenase is the primary component of the enzyme *algD* that is responsible for converting (GDP manose) into (GDP mannuronic acid) (Kamali *et al.* 2020).

The main gene that stimulates alginate synthesis is *algD*, which is followed by the joint action of *mucA* and *algU* (Pulcrano *et al.* 2012).

Because of a modification in the *mucA22* allele, alginate is predominantly generated in the biofilm of mucoid Pseudomonas bacteria. In most CF samples, mucoid forms are present, suggesting that the disease has evolved from severe to persistent (Ciofu and Tolker-Nielsen 2019)

In vitro, one of the main functions of alginate is the scavenging of free radicals generated by macrophages in order to inhibit bacterial clearance by the infected host, and preventing phagocytic clearance, also protecting against defenses through forming a physical barrier which limits phagocytosis, as well as inhibiting neutrophil chemotaxis and complement activation (Moradali *et al.* 2017).

Biofilm formation, phagocytosis and opsonization resistance, and antibiotic diffusion decrease via biofilms have all been discovered to be significant activities of alginate (Strempel *et al.* 2013).

Alginate, which functions as an extracellular matrix in biofilms, protects *P. aeruginosa* from severe circumstances in CF lungs (Wei and Ma 2013).

- **Extracellular eDNA**

Extracellular deoxyribonucleic acid (eDNA) is one among the foremost vital elements of biofilms, and it's discharged once cells are lysed. Under Environmental stress, akin to antibiotic treatment Endolysin Lys is a protein that is expressed inside the F- and R-pyocin cistron regions, has an endolytic action that produces cell lysis (Turnbull *et al.* 2016)

The rod-shaped bacteria rapidly convert into round cells during the planktonic phase, when the cell wall is structurally destroyed, accompanied with lysis. After that, cytosolic proteins, the released eDNA, and especially RNA are encased in vesicles (MVs), that are made up of pieces of damaged cell membrane (Turnbull *et al.* 2016)

eDNA required for microbe attachment and intercellular cohesion (Spagnolo *et al.* 2021), by coordinating cell movement in the previous edge advance rafts, and the twitching motility-movement is promoted by eDNA driven biofilm formation via preserving intact cell alignments (Gloag *et al.* 2013)

Pyocyanin redox cycling produces reactive oxygen species (ROS), which destroy host and pathogen cells and release eDNA (Rada *et al.* 2013)

2.6.2.2 Biofilm stages

P. aeruginosa biofilm formation comprises bacterial attachment, irreversible adhesion, bacteria microcolony growth, the appearance of big structures such as stalks and mushrooms with open pathways demonstrates the maturity of the biofilm, and at the end of the microbial cell dispersion (Thi *et al.* 2020).

Biofilm development represent in five stages: (first stage: primary attachment; second stage: irreversible coherent; third stage: microcolony formation; fourth stage: biofilm maturation; fifth stage: biofilm dispersion).

First stage I: primary attachment: Cell appendages for instance type IV pili and flagella are used by bacteria to attach to surface

In reversible adherence, the bacterial reactions and biofilm development are material specific, according to a proteomic investigation utilizing wild-type PAO1. When *P. aeruginosa* senses and reacts to a particular surface, it leaves traces of the presence of specific bacterial proteins as well as changes in their levels.

Second stage II: irreversible coherent: The transition in bacterial cells from reversible to irreversible adhesion was seen to occur as cell clusters began to form, as evidenced by numerous cells in contact with one another and the substratum, and movement of connected cells halted, as seen microscopically.

Bacterial micro colonies grow in size and confluence, resulting in a more organized phenotype with non-colonized space (Thi *et al.* 2020)

Third stage III: microcolony formation: Microcolonies are the result of the progressive proliferation of connected bacteria into a more organized architecture.

Fourth Stage IV: biofilm maturation: Non colonized gaps are filled by bacteria, which eventually cover the whole surface, as these microcolonies grow into a characteristic of biofilm maturation that occur in the creation of enormous three-dimensional mushroom-like forms.

Fifth Stage V: biofilm dispersion: Cell autolysis disrupts the matrix cavity at the center of the microcolony, allowing dispersed cells to liberate, which then return in a planktonic condition to spread and colonize other surfaces (5days).

Additionally, The major ingredients of the biofilm matrix, is extracellular DNA and also in extracellular polymeric material (EPS) (Das and Manefield 2012).

2.6.3 Type IV pili, flagellum, and fimbriae (swarming via twitching motility)

This bacterium has only one flagellum, which is necessary for bacterial chemotaxis and motility (Costa *et al.* 2015).

Type IV cell membrane pili, contribute in twitching motility movement and crucial for attachment to living cells and other surfaces as well as the retraction and expansion of pili to allow bacteria to move all along host surface.

Cup fimbriae (Cup A, B, C, and E) are four Cup systems that are required for biofilm growth in the early phases, assist in cell-to-cell interaction and the development of microcolonies (Wei and Ma 2013).

2.6.4 LPS lipopolysaccharides

Another cell surface feature is LPS (or endotoxin), which is a crucial component of Gram negative bacteria's outer membrane. It improve adherence.

- Lipopolysaccharide (LPS), that functions as a signaling via toll-like receptors, it has a mechanism of adhesion and consists of three parts:
- Lipid A (endotoxin) that interacts to receptors on host organism to initiate an inflammatory reaction.
- Core oligosaccharide (the CFTR on epithelial cells binds with the core oligosaccharide, resulting in bacterial innate immune system to the infection.
- Side chains of the O-antigen that cause resistance to detergents, regular human plasma and some antibiotics (Huszczynski *et al.* 2020).

2.6.5 Type III secretion (TTS) system

A secretory complicated system (seems to be like a needle) that allow bacterial signaling pathways to penetrate the host cell cytoplasm, causing disrupting host defenses and toxicity.

T3SS causes bacterial dispersion increases and induce cell damage; it has been connected to the acute and invasive pathophysiology diseases like pneumonia (Streeter and Katouli 2016).

2.6.6 Iron chelating

Although iron is a critical ingredient for many bacteria, it is scarce in the early stages of CF lung infection, and Siderophores is a tiny compounds released from bacteria to chelate iron and activate its matrix, evolved to help bacteria import iron. The bacteria *Pseudomonas aeruginosa* form (2) siderophores: pyroverdine and pyochelin. These pigments are potent for bacterial life, in particular through infections as the bacteria's iron supply is depleted by the host.

Iron is required for a variety of biological activities, biofilm formation, DNA replication, Bacterial growth and infection establishment are just a few of the processes involved (Cunrath *et al.* 2020).

2.6.7 Pyocyanin

Pyocyanin is virulence factor and by *P. aeruginosa*, and it induces cell death and oxidative damage in infected species (Das *et al.* 2020).

Pyrocyanin has antibacterial, antifungal, and antiprotozoal propertiesActive *P. aeruginosa* cells generate pyocyanin, a chloroform-soluble blue-green phenazine pigment, Pyocyanin, the organism's major phenazine pigment with antibacterial, antioxidant, and anticancer characteristics, (90 -95)% it is almost found in bacteria strains (Marrez and Mohamad 2020)

It's a tricyclic molecule that belongs to the phenazines family. They are released during the late stationary phase of the medium, giving it a characteristic blue color. It may readily be removed from culture media according to its own solubility in chloroform. 5-methyl-1-hydroxyphenazine undergoes a complicated oxidation-reduction reaction to produce pyrocyanin.

When *P. aeruginosa* synthesizes pyocyanin, it produces reactive oxygen species by interacts with molecular oxygen such as hydrogen peroxide (H2O2). This is the reason of changing in the redox balance of host organism which ends in cell damage and death. (Marrez and Mohamad 2020).

Since eDNA is necessary at all stages of biofilm development, pyrocyanin-mediated eDNA synthesis, which is thought to occur as a result of cell lysis via H_2O_2 generation, might help *P. aeruginosa* biofilm in multiple ways (Marrez and Mohamad 2020).

2.6.8 Other virulence factors

2.6.8.1 Rhamnolipids

P. aeruginosa may produce rhamnolipids, a glycolipid biosurfactant, It has the same hydrophilic and hydrophobic properties as any other bio surfactant. The lipid/fatty acid group and sugar rhamnose combine to form this molecule.

Rhamnolipids (single-rhamnolipids) influence biofilm architecture by helping to maintain biofilm channels and lowering cell adhesion (Wood *et al.* 2018).

2.7 Antibiotic or Multidrug Resistance (MDR)

A Gram-negative pathogen is *Pseudomonas aeruginosa* responsible of acute and chronic infections with impaired immune people systems, such as those with cystic fibrosis or burns (Allegretta *et al.* 2017).

P.aeruginosa the frequent harmful antibiotic-resistant bacteria to human health, as records in the World Health Organization (WHO) (Willyard 2017).

Antibiotics can operate as signaling molecules, lowering or inhibiting the production of virulence factors in bacterial populations, Aside from being efficient at killing or suppressing bacterial proliferation (Kafil *et al.* 2016).

As a result of the rise of multi-drug resistant bacteria, treating *P. aeruginosa* with standard antibiotics has become extremely challenging (Holban *et al.* 2013).

The ability of bacteria to reduce antibiotic efficacy related to functional characteristics or inherent structural indicates intrinsic resistance to these antibiotics (Blair *et al.* 2015).

Resistance can arise as a consequence of mutations or direct transmission of genes encoding resistance mechanisms. Transfer of similar DNA by bacteriophage are techniques for transmitting resistance genes which include Conjugation (the naked DNA direct transfer), transformation (mobile genetic elements via the genes carried by plasmids in direct transfer), and transduction. So mutations or direct transfer of genes encoding resistance pathways can result in resistance (Brauner *et al.* 2016).

The most common source of infection occurs through the element of biofilm ability to withstand drugs and immune system (Vickers 2017).

The tremendous capacity of *P. aeruginosa* to recruit an arsenal of response mechanisms is connected to its incredible survival capability, as well as in clinical settings which appears its ubiquity and longevity, including innate resistance to medicines (Moradali *et al.* 2017).

Three categories comprise: adaptive (biofilm production, latent forms), intrinsic, and acquired (integrons, plasmids, prophages, and transposons (via HGT) can be acquired or mutated), all of that explain the mechanism of Antibiotic resistance, and resulting in varied resistome (López-Causapé *et al.* 2018).

Previous hospitalization, prior antibiotic use and Involvement in an intensive care unit (ICU) are all risk factors for MDR *P. aeruginosa* infection (quinolones, cephalosporins, carbapenems) (Ding *et al.* 2021).

Antibiotic resistance is produced by acquired mutations in antibiotic modulating enzymes or molecular target of them, blockage of entry, efflux pumps, enabling bacteria to resist antibiotic treatment despite of being not embedded in a biofilm (Lebeaux *et al.* 2014).

2.7.1 Persister cells and antibiotcs

The creation of so-called persister cells can also help to acquire antibiotic resistance. Persister cells are bacteria that divide slowly or do not divide at all, making them less susceptible to antibiotics than most bacteria, and they can re-establish infections if antibiotic therapy is stopped.

Persister cells have a lower metabolism, which allows them to avoid antibiotics that target essential biological functions (including translation, cell wall synthesis or replication) (Ciofu and Tolker-Nielsen 2019).

Antibiotic-resistant bacteria known as Persisters are a kind of metabolically inactive, non-replicating bacteria.

Antibiotics those are only effective against metabolically active cells, such as beta-lactams, fluoroquinolones and aminoglycosides, have a significant resistance in Persisters, due to their metabolic dormancy. Antibiotics that kill planktonic cells usually kill persister cells as well (Lebeaux *et al.* 2014).

If the local antibiotic concentration decreases and nutrients become present, Persisters can re-establish infection if they become active (Kim *et al.* 2018).

2.7.2 Efflux pump and antibiotic

Permeability of low outer membrane and Multidrug efflux pumps existence and are two factors involved in increasing the intrinsic resistance by exporting many antimicrobial drugs. the three pumps respectively in *P. aeruginosa* are: MexEF-OprN, MexCD-OprJ and MexAB-OprM (Pang *et al.* 2019).

2.7.3 Antibiotics are classified according to how they work on bacteria

- Cell wall synthesis inhibitors, such as cephalosporins, carbapenems and penicillin, as well as beta-lactamase inhibitors.
- Inhibition of cell membrane activity such as (polymyxins) (colistin).
- Protein synthesis inhibitors including aminoglycosides groups such as gentamycin, and (Glycylcyclines, for eample tigecycline) and chloramphenicol.
- Inhibition of DNA synthesis including Fluoroquinolone group such as ciprofloxacin (Prabhurajeshwar 2019).

2.7.4 Beta (β-Lactam) groups resistance

In many MDR conditions, carbapenems are the last safe therapy choice, The most often utilized therapy options for Pseudomonas infections are β-lactams (Kempf and Rolain 2012).

The most important element is the β-lactamases enzymes formation which capable of hydrolyzing β-lactams (Bush and Bradford 2016).

The ability to create carbapenemase is the most crucial mechanism since most carbapenemase genes are situated on transferable genetic elements and move fast among bacteria (Lari *et al.* 2015).

It comprise four types of β-lactamases: metallo Beta lactamase (MBL) and serine β-lactamases A, C, and D all which hydrolyze Carbapenems (Ismail and Mahmoud 2018).

Because all of β-lactam antibiotics revealed resistant to MBL producing *P. aeruginosa* strains, with the exception of monobactams, and they're linked to high rates of morbidity and death. MBL-coding genes can be found on plasmids and bacterial chromosomes, and they can be passed from bacteria to bacteria via highly mobile genetic components (Farhan *et al.* 2019).

Carbapenemases of the Ambler classes A, B, and D in Gram-negative bacteria are among the carbapenemase classes identified (Karbasizade *et al.* 2015).

Class C -lactamases, it has a high standard of resistance to monobactams, cephalosporins and penicillins.

Most notably *Pseudomonas aeruginosa* phenotypes which are β-lactam antibiotics resistant include: overexpressed efflux pumps, Porin loss, β-lactamases expression, decreased permeability of outer membrane and target changes (Idowu *et al.* 2020).

Due to the deletion of the porin OprD as well as the appearance of carbapenemases *Pseudomonas aeruginosa*, there are two primary pathways of carbapenem resistance. Carbapenem non susceptibility, which is aided in part by plasmids and extrachromosomal elements, reduce the number of antibiotics in treating significant Gram-negative infections (Pang *et al.* 2019).

Infections are treated using antibiotics such as cephalosporins and penicillin, as well as monobactams and carbapenems. All β -lactam antibiotics have 3-carbon and beta-lactam ring, which is the most often used medication class (Arumugham *et al.* 2019).

Beta lactamases are classed A through D (Ambler classification) (Bush 2018).

Metallo β -lactamases are typically connected to resistance genes for aminoglycosides and fluoroquinolones because mobile genetic elements are used to encode them. On the other hand, Except for monobactams like metallo β - lactamase (MBL), aztreonam, causes resistance to all β –lactams (Bassetti *et al.* 2017).

AmpC (class C) is a cephalosporinase-specific broad-spectrum β -lactamase (Bassetti *et al.* 2018).

Potent carbapenem class are hydrolyze by oxacillinases (class D), which are therapeutically beneficial, and also increase resistance (Bassetti *et al.* 2017).

By developing ESBLs and carbapenemases, *P. aeruginos*a obtains horizontally transmitted resistance (Bassetti *et al.* 2018).

Acquired imipenem resistance and impaired meropenem sensitivity can also be caused by inactivation or through the *OprD* a carbapenem-specific porin, is regulated less (Riera *et al.* 2011).

Overproduction of AmpC and inactivation of *OprD*, when combined, can result in resistance to all antipseudomonal β-lactams (Horcajada *et al.* 2019).

2.7.5 Aminoglycosides groups resistance

Aminoglycosides are protein synthesis inhibitors found in bacteria that connect to the 30S component of the ribosome and interfere with the start of protein synthesis. Transferable aminoglycoside modifying enzymes (AMEs) and in rare cases, target changes produce Pseudomonas resistance to aminoglycosides, however it is mostly caused by efflux activity and resrtict outer membrane permeability (Bassetti *et al.* 2018)

Aminoglycoside-modifying enzyme (AME)-make genes are among the most important Gram-negative bacteria resistance genes, particularly in *P. aeruginosa* (Belaynehe *et al.* 2017).

Resistance to aminoglycosides caused by many mechanisms of enzyme modification, although these antibiotics are among the most effective treatments for *P. aeruginosa* infections can be fatal (Odumosu *et al.* 2015).

Aminoglycoside has three enzyme families that target –OH or –NH2 groups (APHs). Because of their specialized targets in the chemical structure, each class has a distinct pattern of action against various aminoglycosides (Bengtsson-Palme *et al.* 2018).

It is found in ocular isolates of *P. aeruginosa* of multidrug-resistant (MDR) that primary source of aminoglycoside resistance is AME genes presence due to the study by (Thirumalmuthu *et al.* 2019).

Aminoglycosides cause mistranslation by attracting the negatively charged 16S rRNA to the 30S subunit's aminoacyl-tRNA site (Becker and Cooper 2013).

Aminoglycosides are frequently used to treat *Pseudomonas aeruginosa* from acute pulmonary exacerbations (PEx) in individuals with juvenile cystic fibrosis (CF) (Ochs *et al.* 2021).

2.7.6 Glycylcyclines (Tetracyclines) resistant

The glycylcyclines are synthetic tetracycline analogs. Only one agent, tigecycline, is currently available for use. tigecycline comprise 9-tert-butyl-glycylamido derivative of minocycline, Tetracyclines and tigecyclines bind to the same ribosome binding site and known to be used in treatment from gram positive and negative bacteria (Liu *et al.* 2020).

2.7.7 Fluoroquinolones resistant

As broad-spectrum antibiotics, fluoroquinolones (FQs) were launched. Because of their high oral bioavailability, are commonly employed in infectious illnesses.

Fluoroquinolones are routinely used to treat pseudomonas ocular infections as a first-line treatment (Khan *et al.* 2020).

In the therapy from cystic fibrosis (CF) infection the Ciprofloxacin and levofloxacin are significant two fluoroquinolone antibiotics, and ciprofloxacin is the last extensively antipseudomonal medication that can be used orally. (Millar *et al.* 2021).

Gene mutations that produce bacterial DNA in *P. aeruginosa* isolates, topoisomerase II and topoisomerase IV are the principal sources of fluoroquinolone resistance. Overexpression of active efflux systems, on the other hand, can decrease membrane permeability. Furthermore, it has been observed that plasmids harboring the ESBL gene can also carry the quinolone resistance gene, and this helps to explain why ESBL-producing bacteria have high levels of significant quinolone resistance (Subedi *et al.* 2018).

2.7.8 Polymyxin resistant

Colistin, or (polymyxin E), is an antibiotic (polypeptide) with several components that belongs to the polymyxin family (Zarate *et al.* 2021).

Pseudomonas aeruginosa infections are commonly treated with polymyxins as a last resort monotherapy (Ahmed 2021).

Polymyxins are polypeptide antibiotics that damage the cell membranes of Gram-negative bacteria by disrupting lipopolysaccharides (LPS), causing increased permeability and, as a result, Cell death is caused by the externalization of cytoplasmic contents (Zarate *et al.* 2021).

3 MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment and appliance

Equipment and appliance used in the study are given in Table 3.1

Table 3.1 Equipment and appliance

Equipment and appliance	Company/Origin
Magnetic stirrer	GallenKamp /England
pipette Variable Volumes (2-20 µL), (0.5-10 µL), (100-1000 µL), (10-100 µL)	Germany
Water Bath	Gallen Kamp /England
Biohazard safety cabinet class II B	Genex / USA
Refrigerator	Concord / Lebanon
Micropipette	Brand/Germany
Deep freezer	Thermo Scirntific/USA
Hot plate with magnetic stirrer	Gallenkamp/UK
Microwave	Gosonic (China)
Compound light microscope	Olympus/Japan
Autoclave	Express/Germany
AURA TM PCR Cabinet	Italy
Microspin 12, High-speed Mini-centrifuge	Bio San/ Germany
V-1 plus, Personal Vortex for tubes	Digsystem / Germany
Distillator	GFL/Germany
Mini-Power Supply 300V, 2200V	Chain
Bio TDB-100, Dry block thermostatbuilt	Bio San / Germany
Electrophoreses	CBS, Scientific / USA
MultiGeneOptiMax Gradient Thermal Cycler	Labnet / USA
Document system	Labnet / USA
Microspin	Biosan / Lativa
Balance	Kernpfb / Germany
Combi-spin	Biosan / Lative
UV.transmission	Vilber lourmat / Farance
Incubation	Jrad / China
Microwave	Gosonic / China
Water distilater	China
Microscope	Novel (China)
Balance	KERN (Germany)
Autoclave	Express(U.K.)
Automatic Elisa reader	PARA medicaly- Italy
Micropipettes	Ependroff(Germany)
Microtiter plate	Nunc(Denmark)

3.1.2 Chemicals

Chemicals used in the study are given in Table 3.2

Table 3.2 Chemicals

No.	Material	Cat #	Company / origin
1	Red safe staining souluion	21141	Intron / Korea
2	Agarose	8100.11	Canada / USA
3	6X Loading dye	21161	Intron / Korea
4	TBE buffer 10 X	IBS.BT004	canada / USA
5	Pre mix pcr	25025	Intron / Korea
6	Ladder 100 bp	24073	Intron / Korea
7	Primer	---	Alpha DNA /canada USA
8	i-genomic BYF DNA Extraction Mini Kit	17045	biotechnology Intron /Korea
9	Ethy alcohol 70%		Tiebah (Iraq)
10	Proteinase K		Intron biotechnology/Korea
11	Crystal violet		Denkapharma(Germany)
12	McFarland tube no.5		bioMérieux, France
13	Glycerol		BDH England
14	Ethanol		BDH England

3.1.3 Medium of culture

Media culture used in the study are given in Table 3.3

Table 3.3 Media culture

Medium	company name /Origin
MacConkey agar	Himedia/ India
King A agar	Himedia/ India
Brain heart infusion broth	Himedia/ India

3.1.4 Standard strains

Pseudomonas aeruginosa ATCC® 27853™ was chosen as a standard strain

3.1.5 Antibiotics cards

Antibiotics used in the study are given in Table 3.4

Table 3.4 Antibiotic cassette (GN76) identification cards (bioMe'rieux) France

Antibiotic group	Antimicrobial agents	CODE	CONCENTRATION
Cephalosporins 1^{st}generation	Cefazolin	CZ	4, 16, 64
Glycylcyclines (Tetracyclines)	Tigecycline	TGC	0.75, 2, 4
Beta lactam/beta lactamase inhibitor combination	Piperacillin /Tazobactum	TZP	2/4, 8/4, 24/4, 32/4, 32/8, 48/8
carbapenems	Imipenem	IPM	1, 2, 6, 12
Cephalosprins 4^{th}generation	Cefepime	FEP	2, 8, 16, 32
Cephalosprins 3^{rd}generation	Ceftazidime	CAZ	1, 2, 8, 32
Aminoglycoside	Amikacin	AMK	8, 16, 64
	Gentamicin	GM	4, 16, 32
Flouroquinolones 3^{rd}generation	Ciprofloxacin	CIP	0.5, 2, 4
Flouroquinolones 2^{nd}generation	Levofloxacin	LEV	0.25, 0.5, 2, 8

3.1.6 Primers

Primers used in the study are given in Table 3.5

Table 3.5 PCR oligonucleotides primers

Primer	Sequence	Tm (°C)	GC (%)	Product size
algD-F	5'- CTACATCGAGACCGTCTGCC - 3'	64	72.35	593 base pair
algD-R	5'- GCATCAACGAACCGAGCATC- 3'	62	70.30	
ps1D-F	5'-TGTACACCGTGCTCAACGAC - 3'	62	70.30	369 base pair
ps1D-R	5'- CTTCCGGCCCGATCTTCATC- 3'	64	72.35	
pelA-F	5'-CATACCTTCAGCCATCCGTTCTTC - 3'	72	73.88	786 base pair
pelA-R	5'- CGCATTCGCCGCACTCAG- 3'	60	71.33	

All primers were used by Alpha DNA/Canada

3.2 Methods

3.2.1 Preparation of cultural media (re-made and synthetic)

According to manufactore instrucion, all media were sterilized plates by autoclave approximately 15 minutes at 15 lbs pressure with (121°C) tempreture

1. Preparation of MacConkey agar media (Wallace *et al.* 1998)

In 1000 mL distilled water we dissolved the medium 28 g of powder, then boiling and autoclaving after cooling, and stored in refrigerator after sterilizing until used.

2. Brain heart infusion broth media preparation

Media are dissolved fully in Distilled water, placed onto sterilized petri plates or tubing and incubated 24 hours at 37°C and according to relevant references (Kayser *et al.* 2005).

3. Preparation of king A agar

To completely dissolve the medium, we put 46.64 g in 10 mL glycerol and 1000 mL distilled water suspension, bring to a boil. To sterilize, autoclave Approximately 15 minutes at 15 lbs pressure (121°C). Aseptically fill sterile Petri plates.

3.2.2 Collecting clinical samples

Burns (40), wounds (30), and sputum (30) were among the (100) specimens gathered from various sources during the period from (October 2021 to the April 2022), sterilized cotton swabs and containers were used to collect microorganisms from medical laboratories for pathogenic examination in order to detect *P. aeraginosa* in Baghdad city in Iraq splitting into three provinces (Al-kindy teaching, Baghdad medical education city, Burns Specialized) Hospitals, the isolates were found (20) *Pseudomonas aeruginosa* using a variety of mediums and chemical properties (APPENDIX 3).

3.2.3 Bacterial isolation and characterization

All specimens were cultivated on MacConkey agar (Riedel *et al.* 2022), and incubated the plates aerobically for 24 hours at 37°C. The *P. aeruginosa* isolate was subcultured on King A agar for confirmatory tests, which included the blue-green pigment pyocyanin, The pure colony of bacteria was then chosen to undergo 64 biochemical tests, including oxidase, catalase, lactose, and additional, as well as confirmation of the isolates' identification using the VITEK2 compact system and device-specific diagnostic kits for *P.aeruginosa*.

3.2.3.1 Bacterial diagnosis by Compact Vitek 2 system (bioMérieux, France):

Vitek 2 system is one of the modern diagnostic systems device It is used in diagnosing bacterial isolates at the species level, and it can also determine the sensitivity of the bacteria to antibiotics, and it works by having special cards containing 64 very small holes, each hole designated for a specific test. It is a large-sized device equipped with two chambers, the first of which is known as the filling chamber, as the holes in the card are filled with bacterial suspension**.** And the second is the incubation chamber, in which the model is incubated to the second day, after which the result is read (Marko *et al.* 2012) (APPENDXX 10) shows this device with its accessories.

followed the steps as per the manufacturer's instructions:

- Wound, burn and sputum swabs were implanted on the culture media (MacConkey's agar) has been set samples are incubated at a temperature (37 celsius) for a period of (24) hours, during which growth was observed on these growth media.

- Dilution was made with a special tube for the manufacturer containing (3) milliliters of distilled water supplied by the company, and a little growth were transferred to it by the standard conveyor.
- The suspension's turbidity was compared to that of a conventional fixed turbidity solution (McFarland) with the turbidity meter supplied by the company which is called the DensiCHEK Plus™, because the ultimate concentration within the tube must be in the range of (0.65-0.5), We repeat the dilution process when measurement result of the sample appears with an inappropriate concentration of the actual percentage specified for turbidity to obtain the correct percentage.
- The tubes were placed in their bearing after adding to each tube a special test strip based on the diagnosis of gram-negative, since gram-negative bacteria have their own strip (GN cassette)).
- The code number affixed to the bearing and to the test tape was entered into the computer attached to the device, through the (smart carrier) accessory device, in order for the device to recognize the bearing and tapes.
- Entering the data of the sample, including giving the sample number, the name of the patient, and the source of the sample (burns–wounds–(sputum) respiratory system).
- Transferring the bearing containing the tubes and their tapes (GN cassette) to the system, then placing it first in the (Filler field), which automatically fills the tapes with bacterial suspension, and once the procedure is finished, the device gives a (transfer instruction).
- The bearing was transferred to the second field, (the reader), who first cut the tapes (GN cassette) and gave a Burden in the form of a digital signal, as it kept the tapes (GN cassette). As for the bearer containing the tubes, it exited the device.
- The tapes were left for (24 hours) at (37°C) temperature, then I read the data of each sample, which is saved on the computer attached to the vitek-2 compact system.
- Incubation and card sealing: Around 35.5+1.0°C, all kinds of card were incubated and each cards was picked once each 15 minutes from the incubators, , these reactions readings were transmitted into the optics system, So its

incubators returned till other moment of reading; These data were gathered at interval of 15 minutes all through the incubating phase.

- Reactions to testing: Raw data calculation are carried out and comparing for every testing to evaluate the reaction. Testing outcomes show as '+'," -', "(–)" or "(+)' of the VITEK 2 Compact.

3.2.4 Pyocyanin assay

King A Agar It is indicated for non-selective Pseudomonas species isolation, culture, and pigment synthesis, The cultures were incubated at 37°C for 24 hours to test the ability of isolated bacteria to produce a pyocyanin pigment, the media contains potassium and magnesium salts in concentrations sufficient to enhance the production of this pigment by supporting its encoding genes and inhibiting the production of fluorescent pyoverdine pigment (Murray and Shea 2004).

3.2.5 Biofilm formation assay

Biofilm cultivation in 96 microplates on brain heart broth (BHI) as described by (Asadpour 2018), And incubated at a temperature of 37°C for 24-18 hours, and after the end of the incubation period, we transfer 3-1 young and pure colonies to test tubes containing 5 mL of saline solution, to form stuck bacteria and compare growth with McFarland bacterial solutions.

180 µL of brain heart broth (BHI) were put into the wells of the plate, after that each well received 20 µL of corrected 0.5 McFarland bacterial solutions. (APPENDIX 9). A lid was put over the inoculation plate, and aerobically incubated under static circumstances for 24 hours at 37°C; gently the plate was cleaned three times with distilled water and set aside to dry at room temperature for 15 minutes (APPENDIX 7).

The plates were shaken in order to remove all non-adherent bacteria. and was stained by 200 microliters of 0.1% crystal violet solution at ambient temperature for 15 minutes ;

after any remaining stains were wiped away with clean water, the plate was left to dry for 20 minutes, the adherent cell was solubilized with 200 microliter of ethanol 95% (APPENDIX 6).

A microplate reader (APPENDIX 8) At 630 nm, the optical density of each well was determined, for data simplification and computation, the classification of bacterial adherence by presented in table 3.6 was utilized, which was based on OD values obtained for individual bacterial isolates (Atshan *et al.* 2012). (Table 3.6).

Table 3.6 Microtiter plate categorization of bacterial adhesion

Mean OD	Biofilm intensity
OD > 4 ODc	Strong
OD <= ODc*	Non – adherent
2ODc> OD> ODc	Weak
4ODc> OD> 2ODc	Moderate

Average OD of the negative control + (3*Standered Deviations) is equal to Cut-off value (ODc).

3.2.6 Antibiotic test susceptibility

The manufacturer's guidelines were followed while utilizing Vitek 2 compact system to assess antibiotic susceptibility in strains isolated of *Pseudomonas aeruginosa* using software version 8.1 and AST-GN76 cards, all of these isolates were examined, and a standard strain (Pseudo ATCC® 27853TM) was utilized as a control.

The AST-GN76 cards provide a set of 19 antibiotics (APPENDIX 11) for assessing drug susceptibility. In this study the profile of antibiotics with various concentrations were employed to determine the sensitivity of Gram-negative bacteria: Cefazolin (4, 16, 64), Tigecycline (0.75, 2, 4), Piperacillin PI /Tazobactum (2/4, 8/4, 24/4, 32/4, 32/8, 48/8), Amikacin (8, 16, 64), Ceftazidime (1, 2, 8, 32), Imipenem (1, 2, 6, 12), Cefepime (2, 8, 16, 32), Gentamicin (4, 16, 32), Levofloxacin (0.25, 0.5, 2, 8), Ciprofloxacin (0.5, 2, 4) as shown in picture.

The broth micro dilution technique, as specified by the Clinical and Laboratory Standards Institute (CLSI), was used to estimate the minimum inhibitory concentrations (MICs) of antibiotics (Patel *et al.* 2015).

3.2.6.1 Vitek 2 compact system for determining the resistance and sensitivity of the bacteria to antibiotics

The AST-GN76 cards were used to evaluate and examine the susceptibility of clinically important Gram-negative bacteria (APPENDIX 11).

Dilution was made with a special tube for the manufacturer containing (2.8) microliters of distilled water supplied by the company, and a little growth were transferred to it by the standard conveyor.

- The suspension's turbidity was compared to that of a conventional fixed turbidity solution (McFarland) with the turbidity meter supplied by the company which is called the DensiCHEK Plus™, because the ultimate concentration within the tube must be in the range of (0.65-0.5), We repeat the dilution process when measurement result of the sample appears with an inappropriate concentration of the actual percentage specified for turbidity to obtain the correct percentage.
- Following the manufacturer's instructions, the cards (AST-GN76 cards) were completely placed, locked, and subjected to a kinetic fluorescence measurement.
- Using integrated vacuum equipment, Manufacturing-quality microorganisms were put into identity cards as well as pure strains of bacterial isolate preparations.
- The bacterial suspension was inserted in a test tube and on a designated rack (cassette), while the AST-GN76 cards were inserted into the correct suspension tube, and the transfer tube was fitted into a neighboring slot.
- Immediately in the compact reader-incubator module of the VITEK 2, the loaded cassette was inserted. The bacterial suspension was injected in small-channels that covered every test well when the vacuum was eliminated and the

air was reintroduced in the port, and infected cards were automatically sealed before being placed on the carousel incubator.

- Whole card were automatically incubated at 35.5°C every 15 minutes, every card was lifted first from incubator carousel and entered into the optical system before being placed back in the incubator until the next read.
- During the incubation phase, data was gathered at 15-minute intervals, and final identification findings were recorded and achieved in less than 24 hours. All of the used cards were immediately discarded in a rubbish can.

3.2.7 Molecular detection of biofilm genes

3.2.7.1 Extraction bacterial DNA

The method for extracting genomic DNA that was utilized by (Isichei-Ukah and Enabulele 2020) with some modification and according to the manufacture instruction by (CBS, Scientific/USA) company:

- Inoculated the *P. aeruginosa* isolates into 5 mL of culture broth and incubated overnight at 37°C till the OD 600 value on a spectrophotometer reaches 0.8-1.0.
- Putting the 2 mL of cultivated bacterium cell in eppendorf tubes and it was harvested through centrifugation at 13.000 rpm for 1 minute and that removed completely the supernatant.
- The bacterial pellet was Re-suspend fully in 300 µL buffer MG with continue mixing the pellet and leftover supernatant by vortex or pipette to get a homogenous solution, The cells were lysed by adding combine of (20 µL Proteinase K, 5 µL RNase A Solution and 300 µL buffer MG) and it must vortex strongly to re-suspend and through incubation for 15 minutes at 65°C with taking in account inverting the tube 5–6 times throughout the incubation period to ensure thorough lysis.

- Adding 250 µL Buffer MB to the lysate after complete lysis, and mix 5 to 6 times by pipetting or gently inverted. Spin down the lid after mixing to eliminate any drips to equilibrate the genomic DNA binding to the column membrane.
- Adding the 250 mL of 80% ethanol further in into lysate and gently invert 5 to 6 times to mix and eliminating any drips by Spin down the lid after mixing.
- Filled a 2.0 mL collection tube halfway pipette with 750 µL of the 80% ethanol that will be placed in the spin column and discard the flow-through and collecting tubes after centrifuging at 13.000 rpm (1 minute).
- Placed the spin column into a new 2.0 mL collection tube (additionally supplied) and Filled the spin column with 700 µL Buffer MW and Centrifuged for 1 minute at 13.000 rpm with make sure of removing flow-through to dry the membrane and centrifuge for another minute to remove any ethanol (reuse the collection tube).
- Placed the spin column into a new 1.5 mL tube (not supplied), and adding directly onto the membrane the 50 ~ 100 µL Buffer ME and Incubated for 1 min at room temperature, and finally centrifuged them at 13.000 rpm for 1 minute elution and getting a pure DNA.
- Using variation of concentration (1.5-2 µL) of the template DNA for polymerase chain reaction (PCR).

3.2.7.2 The *algD* gene primer

The gene primer for *algD* is given in Table 3.7

Table 3.7 The gene primer for *algD*

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'- CTACATCGAGACCGTCTGCC - 3'	64	72.35	593 base pair
Reverse	5'- GCATCAACGAACCGAGCATC- 3’	62	70.30	

3.2.7.2.1 Maxime PCR PreMix kit (20 µlrxn) (i-Taq) (Cat. No. 25025)

It comprises a 2X Master mix solution in addition to a selection of PreMix Kits based on expertise. It is a product that includes all of the components for a single rxn PCR in one tube, including, dNTP mixture, reaction buffer, i-Taq DNA Polymerase. This is the product that gives the best outcomes with the least amount of effort. The first reason is because it includes the whole necessary matrices to conduct PCR, all we need is a primer set, template DNA and D.W. (Table 3.8).

The second element it comprises electrophoresis gel loading buffer, which allows us to conduct gel loading with no need to another process, Because it is done in a quick and straightforward manner, it is suited for a variety of sample applications.

Table 3.8 Maxime PCR PreMix kit (i- Taq) material

Material	**Volume**
DNTPs	2.5 mM
Reaction buffer (10X)	1X
i-Taq DNA Polymerase	5U/µL
Gel loading buffer	1X

- **Diagnosis Gene:** Component used in diagnosis of this gene (Table 3.9)

Table 3.9 Particular encounter gene diagnoses using a mixture

Ingredients	**Concentration**
Final volume	25 µL
Forward primer	10 picomols/µL (1 µL)
Distill water	16.5 µL
Reverse primer	10 picomols/µL (1 µL)
DNA	1.5 µL
Taq PCR PreMix	5 µL

3.2.7.2.2 Optimum condition identification

Following a series of tests to establish the best conditions for primer annealing with supplement, we must adjust The temperature for all samples using (Gradient PCR), These two parameters were regarded critical elements in primer annealing with complement, and the DNA template concentration was varied between (1.5- 2 µL). (Initial denaturation and annealing). (Table 3.10)

Table 3.10 Optimum condition identification

No.	Phase	Tm (^{o}C)	Time	No. of cycle
1-	Initial Denaturation	94^{o}C	5 min.	1 cycle
2-	Denaturation -2	94^{o}C	40 sec	35 cycle
3-	Annealing	60^{o}C	40 sec	
4-	Extension-1	72^{o}C	40 sec	
5-	Extension -2	72^{o}C	7 min.	1 cycle

3.2.7.3 The *pslD* gene primer

The *pslD* gene primer is given in Table 3.11

Table 3.11 The *pslD* gene primer

Primer	Sequence	Tm (^{o}C)	GC (%)	Product size
Forward	5'-TGTACACCGTGCTCAACGAC - 3'	62	70.30	369 base pair
Reverse	5'- CTTCCGGCCCGATCTTCATC- 3’	64	72.35	

- **Diagnosis of Gene**

The same as *algD* gene in paragraph 3.2.7.2.1 and the same optimum condition of detection in paragraph 3.2.7.2.2.

3.2.7.4 The *pelA* gene primer

The *pelA* gene primer is given in Table 3.12

Table 3.12 The *pelA* gene primer

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'-CATACCTTCAGCCATCCGTTCTTC - 3'	72	73.88	786 base pair
Reverse	5'- CGCATTCGCCGCACTCAG- 3’	60	71.33	

- **Diagnosis of Gene**

The same as *algD* and *pslD* gene in paragraph 3.2.7.2.1. and the optimum condition of detection is in Table 3.13.

Table 3.13 Optimum condition identification

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	5 min.	1 cycle
2-	Denaturation -2	95°C	1 min.	35 cycle
3-	Annealing	60°C	1 min.	
4-	Extension-1	72°C	1 min.	
5-	Extension -2	72°C	7 min.	1 cycle

3.2.7.5 DNA electrophoresis

It's a method for sorting macromolecules such as DNA, RNA, and proteins by their size and electric charge, The extraction was carried out using the extraction kit equipped from the American company (CBS, Scientific/USA) and depending on the manufacturer's instructions, the process of extracting DNA from previously diagnosed gram-negative bacterial cells suspension with physiology solution (burns, wounds, respiratory tract) has been done (Tille 2017).

3.2.7.6 Agarose gel preparation

The agarose gel was generated via melting agarose (1.5) g in TBE buffer (100) mL that has already been prepared at 1.5% condensation, due to the research by (Sambrook *et al.* 1989), must boiling agarose before cooling at 45-50 degrees Celsius. In the pour plate, the gel was poured, which had been prepared with an agarose support plate, after the comb had been affixed to produce holes for the samples. The gel was carefully poured and allowed to cool for 30 minutes to avoid air bubbles. The solid agarose has been carefully scraped from the comb.

3.2.7.7 Sample preparation

We do the next steps to prepare for electrophoresis due to (Tille 2017) with some modifications:

- Mixed 5 µL of the supposed DNA with 3 µL of the processor loading buffer (Intron / Korea) to be electrophoresis (loading dye).
- Poured the loading process into the gel pores and exposed the loading process to 70 vcm^2 electric current for 1-2 hours, till tincture crossed the gel's other side.
- Position the gel in pool comprises 500 mL of distilled water and 30 µL Red safe Nucleic acid staining solution and tested the gel by a UV source with a wavelength of 336 nm (Figure 3.1).

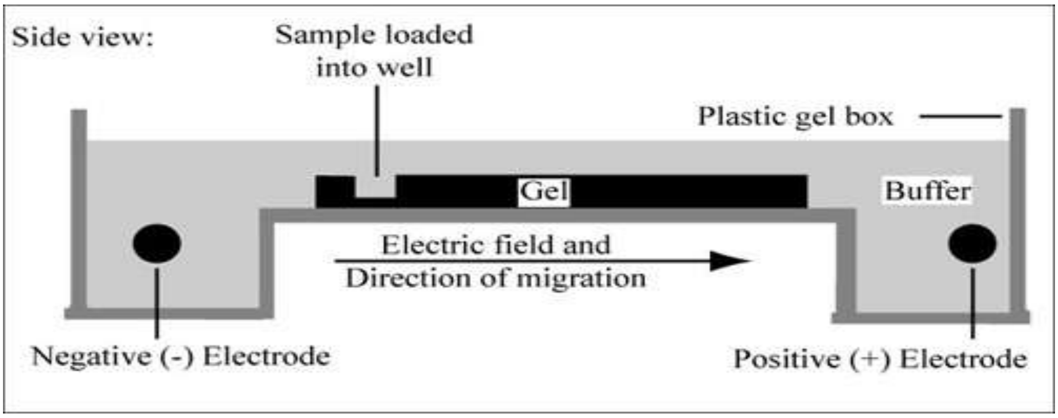


Figure 3.1 **Working the electrophoresis system**

3.2.7.8 Solution for (red safe) staining nucleic acids

RedSafe (20.000x) Nucleic Acid Staining Solution is a novel and suitable substitute of ethidium bromide (EtBr) staining. it emits a green fluorescence by bounding to DNA or RNA; and through connecting to nucleic acid, The fluorescence excitation maxima of this new dye are as follows: one at 309 nm and the other at 419 nm. An excitement may also be detected at 514 nm.

At a wavelength of 537 nm, Red Safe bound to DNA fluoresces. RedSafe Nucleic Acid Staining Solution's sensitivity (20.000 x) is equivalent to EtBr. This value staining (20.000x) were used by each of EtBr and Red Safe Nucleic Acid Staining Solution which in return induces a significant reduction in mutations than EtBr (putent mutagen) in the Ames test.

3.2.7.9 Specific primers for use in the procedure

IDT (Canadian firm Integrated DNA Technologies) investigated the primers after they had been lyophilized, As a stock solution, to reach a final concentration of 100 pmol/µL it is dissolved in free ddH_2O, and maintained as stock at -20°C to create an active primer suspension concentration of 10 pmol/µL, to get a final capacity of 100 µL, this was discovered by dissolving the stock solution (10 µL) in free ddH_2O water (90 µL).

3.3 Data and Static Analysis

Statistical package SPSS-22 program used for Data analysis, in which Simple percentage measurements were reflected. The significance of the difference in percentages was determined using the Chi-square test (X-) (quality data), cross table, one sample, Z-test, and the Binomial test was used to differentiate between the frequencies. When the P-value for the relevance check was equal to or less than the P-value for the main study, statistical significance was considered (0.05).

4 RESULTS AND DISCUSSION

4.1 Bacterial Isolation and Identification

Standard identification procedures used to be able to recognize twenty (20%) *P. aeruginosa* isolates from all clinical samples, including colony characteristics on MacConky agar, the result was brownish green coloration after incubation at 37°C for an overnight period and irregular feathered edges, grape- like odor (amino acetophenone) and approximately 2 mm in diameter and lactose non fermenting (Luis *et al.* 2020) (APPENDIX 12), with using the Kings A agar media to test the production of pyocyanin, The findings of biochemical tests show negative reactions for methyl red, lactose and Indol, and positive reactions for catalase, gelatin hydrolysis, citrate utilization, and oxidase, the capability of VITEK®2 in recognizing *P. aeruginosa* is (99-98 %) (It indicates excellent). (APPENDIX 2).

Each card in the data base is based on the positivity of biological processes, with VITEK2 compact system confirmation of all isolates.

P.aeruginosa isolates were found in the highest number at Burns, with a ratio of (35 %). This might be due to the pathogen's various potentially virulent components, including as protease, pyocyanin, and hemolysin, which aid in host cell attachment, tissue damage, and defense system disruption, allowing it to penetrate and infect human tissues.

Due to the breakdown of the skin barrier and frequent scrubs of the burn site, Pseudomonas cross-contamination is more prevalent in burn patients with high resistance to topical povidone iodine (Riedel *et al.* 2022), the percentage of wounds infections were (15.5%) and the rate in (sputum) infection (17%), it indicates that *P. aeruginosa* can colonize a variety of human locations like (mucous membrane, respiratory tract). It has been shown to induce morbidity in humans, particularly in those with compromised host defenses (Riedel *et al.* 2022) (Table 4.1).

Table 4.1 Percentage of bacterial isolates among sources

Types of isolates	Collected isolates	Isolates number	%
Burns	20	7	35
Wounds	45	7	15.5
Sputum	35	6	17.1
Total	100	20	67.6%

4.1.1 Prevalence of *P. aeruginosa* in clinical samples isolated from Baghdad hospitals

The result showed age and gender distribution of patients from whom clinical samples of *P. aeruginosa* as in Table 4.2, Table 4.3, Table 4,4 and Table 4.5 respectively. Frequency of infection was highest in males (60%) than females (40%), also found wound infections in males was high (41.66%) while in female the high infection was in sputum and burn infection (37.5%)

The study showed that most infection occurred in the ages between (41-50) by the rate (40%) with sputum infections which was the highest infection, the least infection was (25%) among age between (30-40). The middle age of the patients was by the age (51-71) (rate 35%). In addition, in blood group types (O+,AB+,A+,B+, B-, O-,A-), the highest infection rate was in blood type (O+) (35%) and the lowest infection rate was in blood types(B-,O-,A-) (5%).

Table 4.2 Frequency of the gender due to sources of *P. aeruginosa* isolates

Gender	Isolates type			Frequency (n)	Relative frequency (%)
	Wound	Burn	Sputum		
Male	5	4	3	12	60%
female	2	3	3	8	40%
Total	7	7	6	20	100%

Table 4.3 Distribution the gender (males) due to sources of *P. aeruginosa* isolates

No.	Sample type	Sex	Frequency (n)	Relative frequency (%)
1	Wound	Male	5	41.66%
2	Sputum	Male	3	25%
3	Burn	Male	4	33.33%
Total			12	100%

Table 4.4 Distribution the Gender (females) among sources of *P. aeruginosa* isolates

No.	Sample type	Sex	Frequency (n)	Relative frequency (%)
1	Burn	Female	3	37.5%
2	Sputum	Female	3	37.5%
3	Wound	Female	2	25%
Total			8	100%

Table 4.5 Age distribution of *Pseudomonas aeruginosa* isolates

Age	Sample type			Frequency (n)	Relative frequency (%)
	Wound	Burn	Sputum		
30-40	3	1	1	5	25%
41-50	2	2	4	8	40%
51-71	2	4	1	7	35%
Total	**7**	**7**	**6**	**20**	**100%**

Table 4.6 Frequency of *Pseudomonas aeruginosa* isolates among blood groups

Blood type	Frequency (n)	Relative frequency (%)
O+	7	35%
A+	3	15%
AB+	5	25%
B+	2	10%
A-	1	5%
O-	1	5%
B-	1	5%
Total	20	100%

4.2 Pyocyanin assay results

P. aeruginosa 20 isolates were tested for pyocyanin production, the results shows at the beginning of culture that some of samples has color (blue-green) which refers to pyocyanin, but after that it transient and does not exhibit the same pigments in these strains, and that may related to multifactorial mechanisms comprise (repeating culture of bacteria which impair the ability of producing pyocyanin) or (inhibiting one of the genes responsible for phenotypic or genotypic type). the genes controlled the expression of phenotype is (*phzS, phzM*) and contribute with it the *phzA* gene which is the main responsible for production and its genotype, all of these genes help in phenotypic expression for this pigment, and any inhibiting in these genes may result in not producing pyocyanin, The irregularities in the production of this dye are due to the fact that they act as antibiotics, as they adversely affect a number of human cell functions, the most important of which are the growth of respiratory cells and skin cells, Calcium balance and also the release of interculin. (Shah *et al.* 2016). (APPENDIX 13).

And there are some strains known to be not producing pyocyanin in their common mechanism, and those strains possibly one of them, in addition the presence of phzS and NADH is necessary for producing Pyocyanin, lack in any of them disturb the physical transition or transient of producing the pyocyanin (Gonçalves and Vasconcelos 2021).

4.3 Evaluation the Susceptibility of Antibiotics Among Isolates

The results of bacterial resistance isolates to antibiotics showed in Table 4.7 due to the VITEK 2 Compact system with AST-GN76 cards, that showed the highest resistance was against Tigecycline (8) the result similar to (Kim *et al.* 2018, Keyal *et al.* 2020), followed by Cefazolin (64), result was close to (Ali *et al.* 2020) and show high sensitive to Ciprofloxacin (0.25) the same result as (Abdallah and Gabur 2021) and (Faiz *et al.* 2022), followed by Levofloxacin (0.5) the result close to (Ali *et al.* 2020) and Gentamicin (1), cefepime range (1), Ceftazidime (2) and Imipenem (2) respectively, the results relatively the same as (Abdallah and Gabur 2021, Faiz *et al.* 2022), Amikacin (2) and Piperacillin /Tazobactum (8) the result was similar to (Faiz *et al.* 2022) depend on MICs outcomes of each antibiotics. (Table 4.7).(APPENDIX 1).

Cefazolin is belong to cephalosporins group and Cephalosporins is a Cell wall synthesis inhibitors (Prabhurajeshwar 2019), and consider an MDR *P. aeruginosa* infection due to previous hospitalization, prior antibiotic use and Involvement in an intensive care unit (ICU) are all risk factors for increasing resistance to this antibiotic (cefazolin) (Ding *et al*. 2021).

According to a study from India, over half of *Pseudomonas aeruginosa* bacteria are resistant to cephalosporin medicines. (Molla *et al*. 2020)

Furthermore, a comprehensive evaluation of antibiotic resistance in Bangladesh published in 2019 indicated that most infections had significant levels of resistance and that typical first-line antimicrobial medicines were ineffective in most cases (Ahmed *et al.* 2019).

The results also indicates the resistance to tigecycline and that approved by (Cui *et al.* 2021) which explain the role of mutation in the molecular mechanism through a newly found high-level tigecycline resistance mechanism mediated by plasmids Tet (X), and according to (Riedel *et al.* 2022). It has poor antimicrobial action against *P. aeruginosa*.

When such incidents happen (spontaneous mutations in essential chromosomal genes, transfer of entire new genes or groups of genes from another species), the end result can range from minor changes in microbial sensitivity that can be addressed with higher dosages of the medicine to total loss of sensitivity. Typically, spontaneous random mutations in bacterial populations cause chromosomal drug resistance.

The possibility of such a mutation being helpful is limited, and the potential of giving drug resistance is even rarer. Despite this, such changes do occur due to the enormous number of microorganisms in each population and the constant process of mutation (Kathleen 2017).

Due to the findings of our study, fluoroquinolones demonstrate susceptibility. This is due to their involvement in binding to DNA gyrase and a similar enzyme, and this mechanism of action assures that fluoroquinolones provide broad-spectrum efficacy. Ciprofloxacin and levofloxacin are two most frequently prescribed quinolone antibiotics for urethritis, gastrointestinal infections, sexually transmitted disease, respiratory infections, osteomyelitis and soft tissue infections. Levofloxacin is especially recommended for bronchitis, pneumonia, and sinusitis (Kathleen 2017).

Cefepime and ceftazidime are broad-spectrum cephalosporin drug which has high antibacterial activity against a broad range of Gram-negative bacteria, particularly *Pseudomonas aeruginosa*. (Gallagher and MacDougall 2016).

Carbapenems are broad-spectrum antibiotics and a cell wall sunthesis inhibitors that have a similar mechanism of action to penicillin but are more resistant to beta-lactamases, making them an ideal therapy option. They work at very low concentrations and may be taken by mouth with just little side effects (allergies, for example). Imipenem inhibits a huge spectrum of Gram-positive and negative bacteria, Gram-negative rods and anaerobes, whereas Meropenem kills more Gram-negative bacteria (Riedel *et al.* 2022).

Gentamicin and Amikacin belong to the aminoglycoside group and aminoglycoside is an inhibitor of protein synthesis.

Gentamicin is a less toxic antibiotic commonly used to treat gram-negative rod diseases; Amikacin is a new aminoglycoside that is used to treat gram-negative infections (Riedel *et al.* 2022).

Piperacillin /Tazobactum are beta-lactamase inhibitors which resemble beta-lactams structurally and bind to various beta-lactamases irreversibly, preventing them from inactivating the co-administered beta-lactam.

As a result, piperacillin/tazobactam inhibits *P. aeruginosa* growth. (Gallagher and MacDougall 2016).

As a consequence, these antibiotics might be helpful in hospitals, to treat *P. aeruginosa* infections, especially in individuals with weakened immune systems, such as those who are admitted to the burn unit or those with wounds or tracheal infections (Riedel *et al.* 2022).

By (Riedel *et al.* 2022) all of (Aminoglycoside + carbapenemes + an antipseudomonal penicillin), it was the medication of choice for Suspected or Proven Microbial Pathogens, Table 4.7 explain the MICs results of the antibiotic and the activity (resistance or susceptible) for each antibiotics. The quality control organism was *Pseudomonas aeruginosa* ATCC® 27853TM. (for comprehensive quality control) and To classify organisms as susceptible or resistant, the (CLSI) or (the Clinical and Laboratory Standards Institute) standards were used to compare the results. (Figure 4.1).

Table 4.7 MICs results of antimicrobial agents

Antimicrobial agents	CODE	ACTIVITY	MICs
Cefazolin	CZ	R (resistance)	>=64
Tigecycline	TGC	R(resistance)	>=8
Piperacillin PI /Tazobactum	TZP	S (susceptible)	8
Amikacin	AMK	S (susceptible)	<=2
Ceftazidime	CAZ	S (susceptible)	2
Imipenem	IPM	S (susceptible)	2
Gentamicin	GM	S (susceptible)	<=1
Cefepime	FEP	S (susceptible)	<=1
Levofloxacin	LEV	S (susceptible)	0.5
Ciprofloxacin	CIP	S (susceptible)	<=0.25

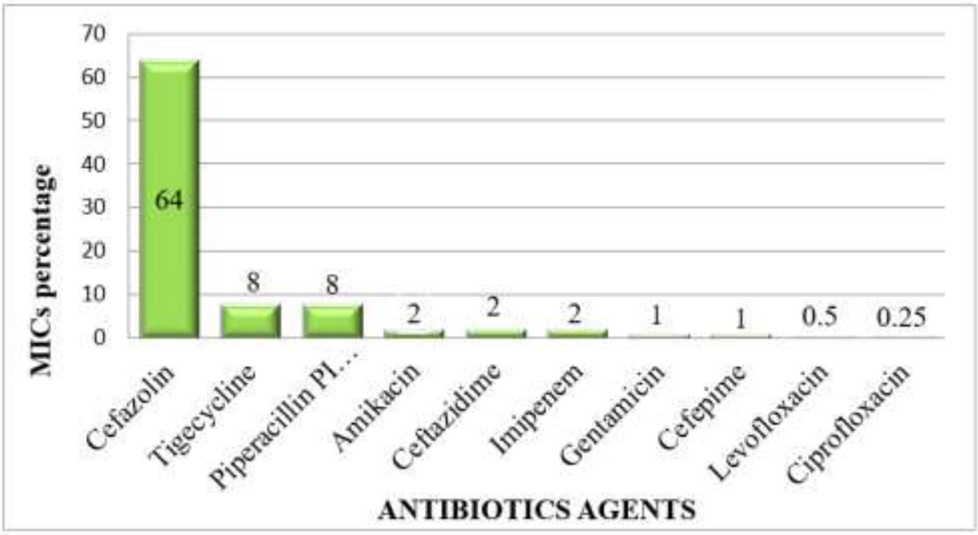


Figure 4.1 MICs percentage of antibiotics

4.4 Detection of Biofilm Characteristics in Clinical Isolates

Biofilm production was detected using the Micro titration plate technique, with findings showing that out of 20 isolates, biofilm in *P.aeruginosa* growth was divided as strong, moderate and weak. (APPENDIX 4), under a set of experimental situation by using spectrophotometer technique for the qualitative biofilm formation assay, Due to OD values, the determined of cut off value (ODc) was after calculating the biofilm forming ability for each bacterial isolate and the negative control. The three standard deviations (SD) higher than the negative control's mean OD. (Table 4.8 biofilm formation based on estimated cut off value of this study). (Al-Fridawy *et al.* 2020) (Table 4.8).

Table 4.8 Biofilm results in microtiter plate according to optical density

Biofilm formation	Range of optical density (OD)
1- non biofilm producer	<0.220
2-weak	0.220-0.44
3-moderate	0.44-0.88
4-strong	>=0.88

Cut off value = 0.220 (defined as the mean of control OD 630 plus 3 standard deviation).

The majority of strains (50%) produced moderate biofilms, 35% produced strong biofilms, and 15% produced weak biofilms. This proportion of production of biofilms was close to the outcome of (Divyashree *et al.* 2022) which found 39% of strong biofilm and 57% for moderate biofilm producers, while the result of producing weak biofilms (15%) was close to (Al-Fridawy *et al.* 2020) (13%) results. (Table 4.9) (Figure 4.2).

The study found that 100% of the entire 20 isolates form biofilm, indicating that the bacteria have a strong capacity to form biofilms, and that this ratio is larger than the formation seen in the previous study by (Mubarak 2021) were they recorded that *P.aeruginosa* form 94.28% biofilm production and 5.71% not production. This clarifies the quorum sensing system function in controlling the gene expression in bacterial cell which permit the biofilm forming under un appropriate condition and otherwise it effects the pathogenicity of bacteria by controlling the biofilm formation and other virulence gene (Christiaen *et al.* 2014).

Table 4.9 Biofilm intensity frequency

BIOFILM Production	Frequency	Relative frequency (100%)
Strong	7	(35%)
Moderate	10	(50%)
Weak	3	(15%)
Total No. (%)	20	(100%)

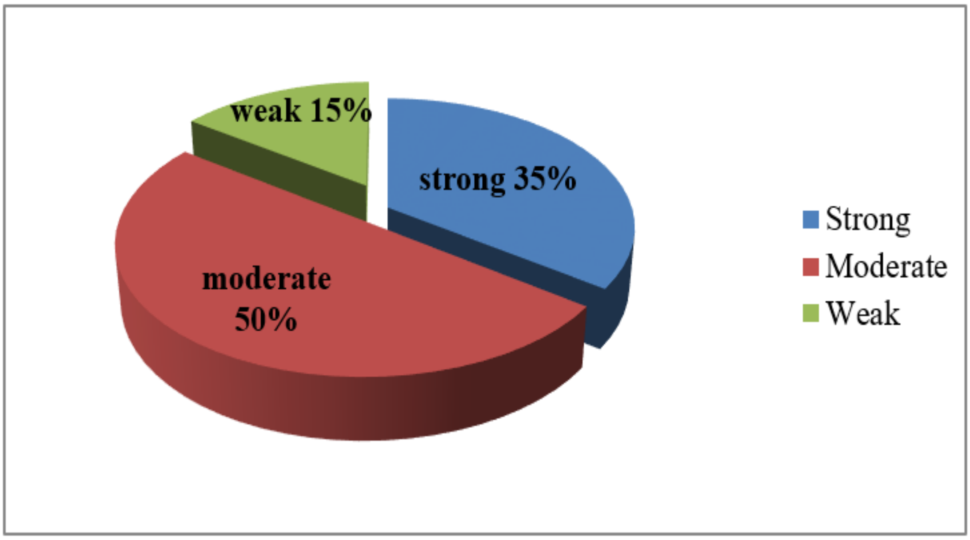


Figure 4.2 Biofilm production frequency

There might be differences in biofilm growth between isolates attributable to a number of variables, considering the ability of each isolate to create biofilm, the quantity and quality of quorum sensing signaling molecules, as well as the primary number of adherent cells, produced by each isolate. Among specimens with moderate biofilm, there is no clear pattern governing the distribution of biofilm intensity. It suggests that biofilm intensity is a specimen-dependent variable. probably the genetic material of each strain explains the observed differences (Abd Al Rhman *et al.* 2018).

4.4.1 Distribution of biofilm intensity among site of infection

Isolates from wounds were divided into strong 42.85%, moderate 42.85%, and weak 14.3%, respectively, according to biofilm producers. In addition, isolates collected from burns had 28.6% strong and 71.4% moderate biofilm ability, while isolates from the sputum region had 33.33% weak, moderate and strong biofilm ability. (Table 4.10),

In a burn infection, there are no weak biofilm producers. This might be because of the pathogen's capacity to create biofilm. (levels of metabolic activity inside the biofilm), and their strong and moderate biofilm intensity may be attributed to the burned percentage and depth partial thickness of burn wounds (Ramakrishnan *et al.* 2016).

(APPENDIX 3). Chi-square analysis showed the same p-value (< 0.001) for burns and wound infections, while p-value (1) during respiratory infection, total p-value (0.4) for biofilm density in all samples that indicates positive results because it is less than the standard p value (0.05), and chi-square (3.65).

It shows high biofilm density in both burns and wound infections, while less biofilm density in sputum infections (Figure 4.3).

Table 4.10 Distribution of biofilm intensity among clinical isolates

SAMPLE TYPE	BIOFILM INTENSITY			
	Strong	**Moderate**	**Weak**	***P value***
WOUND	3 (42.85%)	3 (42.85%)	1 (14.3%)	<0.001
BURN	2 (28.6%)	5 (71.4%)	-	<0.001
SPUTUM	2 (33.33%)	2 (33.33%)	2 (33.33%)	1.00
Total No. (%)	7 (35%)	10 (50%)	3 (15%)	
Chi-square	3.65			
P value <0.05 significant	0.455			

* These statistical results are obtained by the chi-square test.

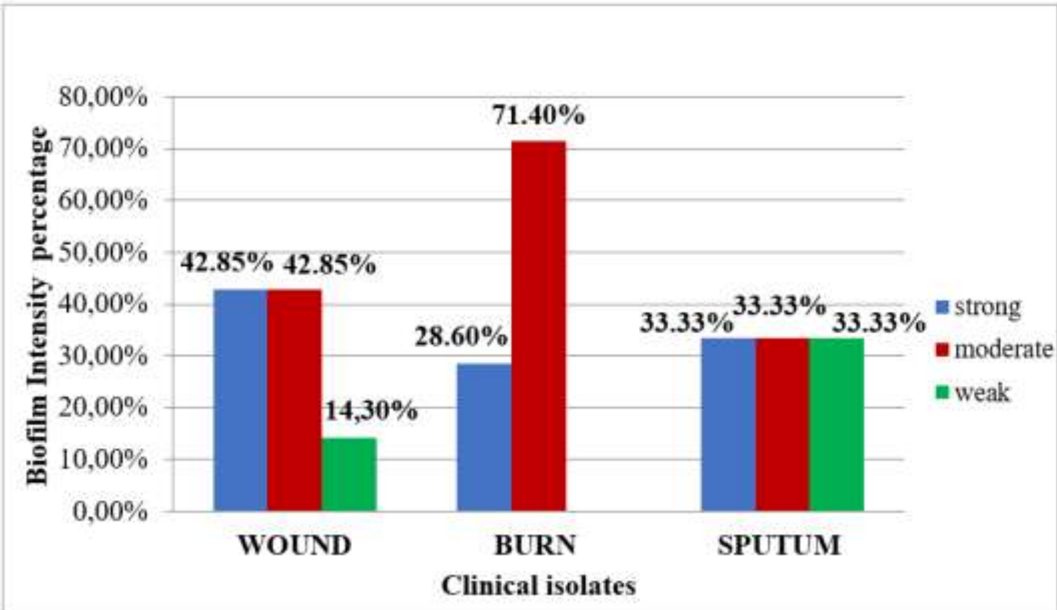


Figure 4.3 Distribution of biofilm intensity among clinical isolates

4.4.2 Biofilm genes Detection (*algD*, *pelA* and *pslD*) in clinical isolates:

Instead of EtBr, we utilize RedSafe Solution (20.000x) to identify nucleic acid on agarose gels. (Item number 21141 in the catalog) (Figure 4.4) (Figure 4.5) (Figure 4.6) (Figure 4.7) (Figure 4.8)

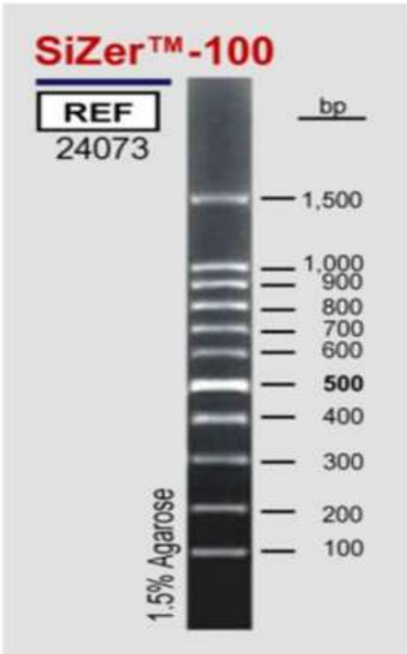


Figure 4.4 SiZer DNA markers (intron)

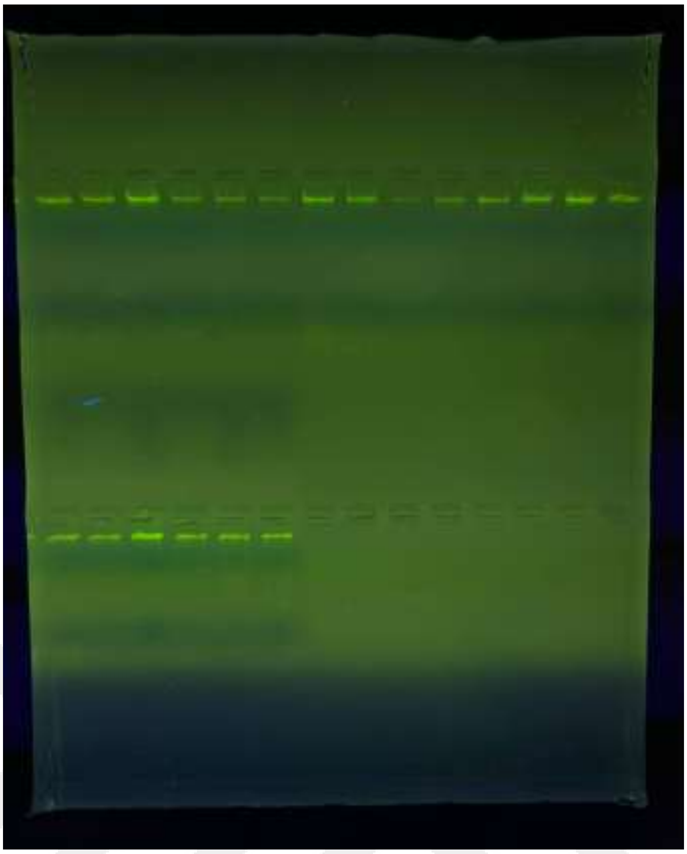


Figure 4.5 Samples (1-20) genomic DNA extraction from bacterial samples

- ***algD* gen result**

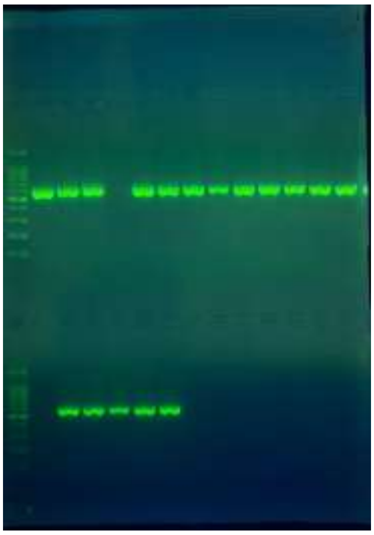


Figure 4.6 Shows a PCR result with a 593-bp band size

Electrophoresis on 2% agarose at 70 volt/cm2 produced the final product. 1 hour of 1x TBE buffer N stands for DNA ladder (100)

- ***pslD* gen result**

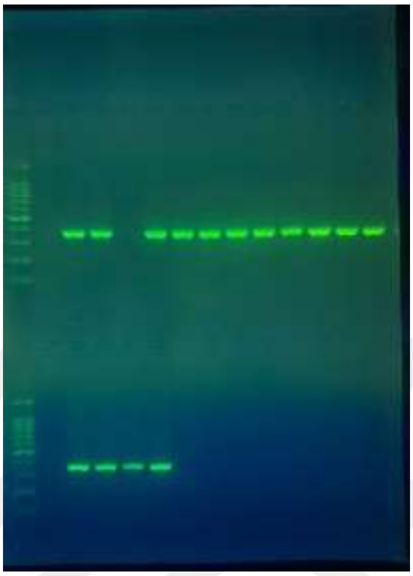


Figure 4.7 The band size of the PCR result is 369 bp

Electrophoresis on 2% agarose at 70 volt/cm2 produced the final product.(1 hour of 1x TBE buffer). N: DNA ladder (100).

- ***pelA* gen result**

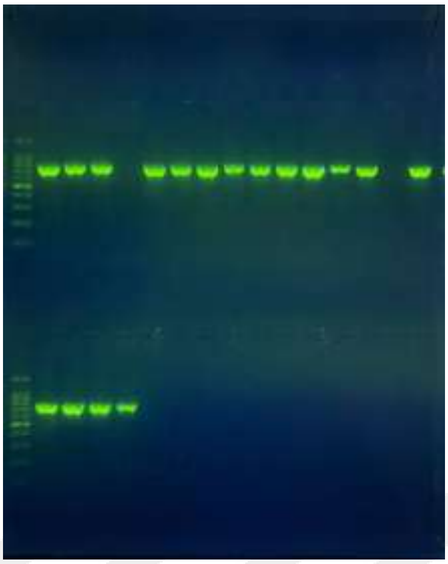


Figure 4.8 Shows a PCR result with a 786-bp band size

Electrophoresis on 2% agarose at 70 volt/cm2 produced the final product. 1 hour of 1x TBE buffer N stands for DNA ladder (100)

4.4.3 Distribution of virulence genes among various infection sites

This study looked at the percentages of several *P. aeruginosa* virulence genes (*algD*, *pelA*, and *pslD* genes) and their connection to infection site and found positive activity was highest in sputum (100%), next pus from wounds (80%), and finally burns (75%) (table 4.11).

All genes had the same positive activity in burn infections 71.4%, and in respiratory infections all genes had 100% positive roles, while *algd* and *pelA* had the same positive role in wound infection 85.7%, these are close results of *algD* gene in respiratory tract infection (Al Dawodeyah *et al.* 2018) (98%) and results close to 87% of *algD* genes of burn infection in the study (Ghadaksaz *et al.* 2015), except for the *pslD* gene, which had less favorable activity (57%) of outcomes in wounds, reflecting the weak role of this gene in wound infection, and Chi square analysis revealed same p value results (<0.001) in all isolate, that indicates high prevalence of (*algD*, *pelA* and *pslD* genes in all type of samples and that agree with the study results. (Table 4.11) (Figure 4.9).

Table 4.11 The percentages of virulence genes discovered among various infection sites

Genes	The various infection sites			
	Burn	**Wound**	**Sputum**	***P value***
algD Positive No. (%) negative No. (%)	5 (71.428%) 2 (28.571%)	6 (85.7%) 1 (14.2%)	6 (100%)-	<0.001
pelA Positive No. (%) negative No. (%)	5 (71.428%) 2 (28.571%)	6 (85.7%) 1 (14.2%)	6 (100%)-	<0.001
pslD Positive No. (%) negative No. (%)	5 (71.428%) 2 (28.571%)	4 (57.142%) 3 (42.857%)	6 (100%)-	<0.001
Total No. (%)	7 (75%)	7 (80%)	6 (100%)	<0.001
P value	0.001	0.001	1	
P value <0.05 significant				

*This statistical results are obtained by chi-square test.

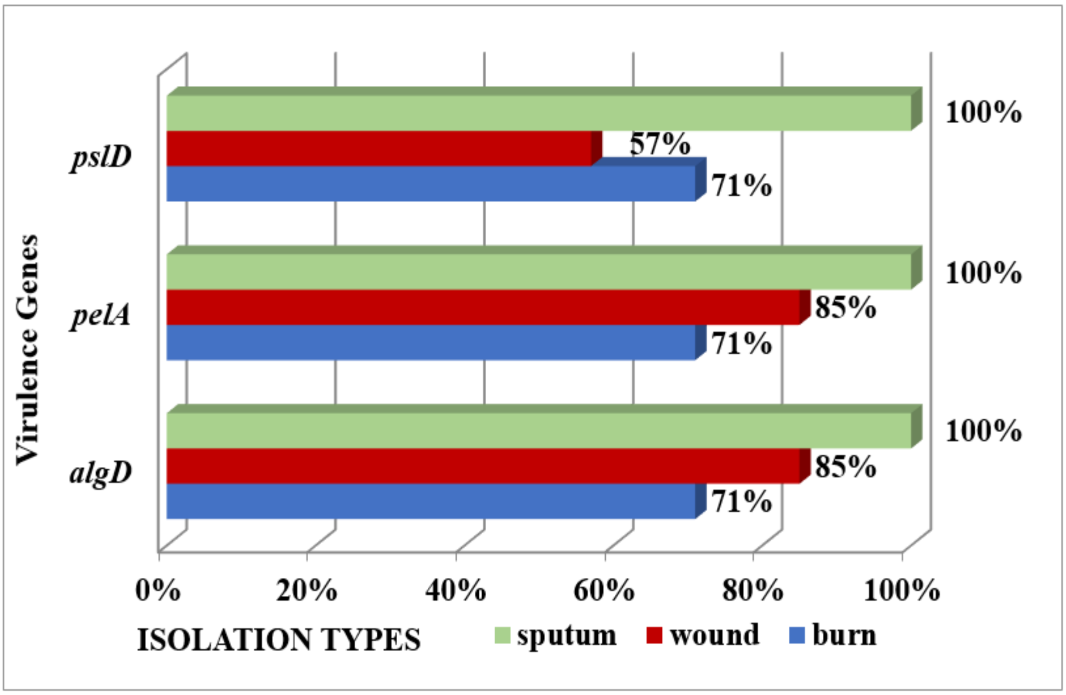


Figure 4.9 The percentages of virulence genes discovered among various infection sites

It is known that the main gene that stimulates alginate synthesis is *algD*, which is followed by the joint action of *mucA* and *algU*. (Pulcrano *et al.* 2012) and the GDP mannose dehydrogenase is the primary component of the enzyme *algD* that is responsible for converting (GDP manose) into (GDP mannuronic acid) (Kamali *et al.* 2020).

The gene *algD* was detected in 17 isolates (85%) and other studies reported close results as (89%) by (Elmaraghy *et al.* 2019, Karballaei Mirzahosseini *et al.* 2020) The *algD* gene was confirmed to be present in all of the isolates, which reffer to the significant ability for alginate in biofilm formation, interfering with the antibiotic response of *P.aeruginosa* isolates (Karballaei Mirzahosseini *et al.* 2020) (Table 4.12).

Also this prevalence reflect the main function of alginate in Biofilm formation, phagocytosis and opsonization resistance, and antibiotic diffusion decrease (Moradali *et al.* 2017).

This conclusion is consistent with the majority of findings showing alginate that is still pertinent in the subject of *P. aeruginosa* infection of CF lung mucoid strains, explaining the function of alginate production and control in chronic CF lung infection (Mahajan *et al.* 2021).

pelA is an oligogalacturone present in 17 isolates (85%) essential for Pel biosynthesis and biofilm structure, which is close to the results recorded by (87%) (Elmaraghy *et al.* 2019).

pslD is a type of protein secretion involved in the exopolysaccharide export process, which makes it necessary for biofilm development (Kamali *et al.* 2020). The rate of the presence of *pslD* gene found in 15 isolates is (75%), and this result relatively the same as (73.8%) due to (Alrawi and Mahmood 2022).

Table 4.12 Virulence gene percentages in isolated infections

No.	Genes	Positive No. (%)	Negative No. (%)
1.	*algD*	17 (85%)	3 (15%)
2.	*pelA*	17 (85%)	3 (15%)
3.	*pslD*	15 (75%)	5 (25%)

4.5 Biofilm Development and the Presence of Biofilm Genes

The most prevalent virulence genes found in all 20 isolates were *algD* and *pelA* (85%), followed by *pslD* (75%), the results of *algD* and *pelA* (85%) in biofilm producing bacteria were comparable to the results found of the *algD* frequency was 87% by (Ghadaksaz *et al.* 2015), and also in the study by (Elmaraghy *et al.* 2019) the frequency of *pelA* were 82.6% which is close to *pelA* frequency in our study, (Table 4.13). Binomial test was used and the p value (<0.001) in all biofilm genes which proved high production of biofilm with related biofilm genes

Table 4.13 The frequency of *pelA* and *pslD* and *algD* genes among biofilm producer strains

Genes	BIOFILM FORMATION	*P value*
	Biofilm producer n=	
algD: positive No. (%)	17 (85%)	<0.001
Negative No. (%)	3 (15%)	
pelA: positive No. (%)	17 (85%)	<0.001
Negative No. (%)	3 (15%)	
pslD: positive No(%)	15 (75%)	<0.001
Negative No. (%)	5 (25%)	
Total No. (%) positive	17 (82%)	<0.001
(%) negative	11 (18%)	
P value <0.05 significant		

*Binomial test is used to differentiate between the frequencies

4.5.1 Clinical isolates' genotypic patterns and biofilm development

Upon the appearance of these genes, 3 genotypic patterns were found, the most frequent pattern n=15 (75%) All three *algD*, *pslD*, and *pelA* genes were present in all isolates a similar time (as *algD* +/*pslD* +/*pelA* + genotypic pattern), 10% (n=2) found there are just two genes *algD* and *pelA* (as *algD* +/*pslD* -/*pelA*+), the third pattern did not harbor any genes 15% n=3 and (identified as *algD* - /*pslD* - /*pelA* –). It revealed a significant occurrence rate of *algD*, *pslD* and *pelA* genes in which is close to the result (75%) of (Kamali *et al.* 2020) (73.8%) (APPENDIX 5).

The Chi-square is done by cross table and p value was performed for comparison between biofilm intensity and biofilm genes by one sample. (Table 4.14).

On the other hand, the observation of biofilm formation although no genes were detected in these isolates indicated that *P. aeruginosa* could form biofilm despite gene deficiency and could be associated with the presence of an alternative biofilm genetic factor that plays a role in matrics development, in addition may other genes have not yet been investigated, play a function in biofilm creation (Moradali *et al.* 2017) (Figure 4.10).

Table 4.14 Genotype and phenotype biofilm distribution

Genotypic biofilm Pattern, No. (%)	**Phenotypic biofilm pattern, No. (%)**			**Total No%**
	Strong	**Moderate**	**Weak**	
***algD* +/*pslD* + /*pelA* +**	6 (30%)	7 (35%)	2 (10%)	75%
***algD* +/*pslD* - /*pelA* +**	1 (5%)		1 (5%)	10%
***algD* - /*pslD* - /*pelA* -**	-	3 (15%)	-	15%
P value	0.635	0.192	1.00	
Total No. (%)	7 (35%)	13 (50%)	3 (15%)	100%
Chi-Square	**2.39**			
Total p value	**0.764**			

*This results are obtained by performing the Z-test by considering the positive results as indication of success.

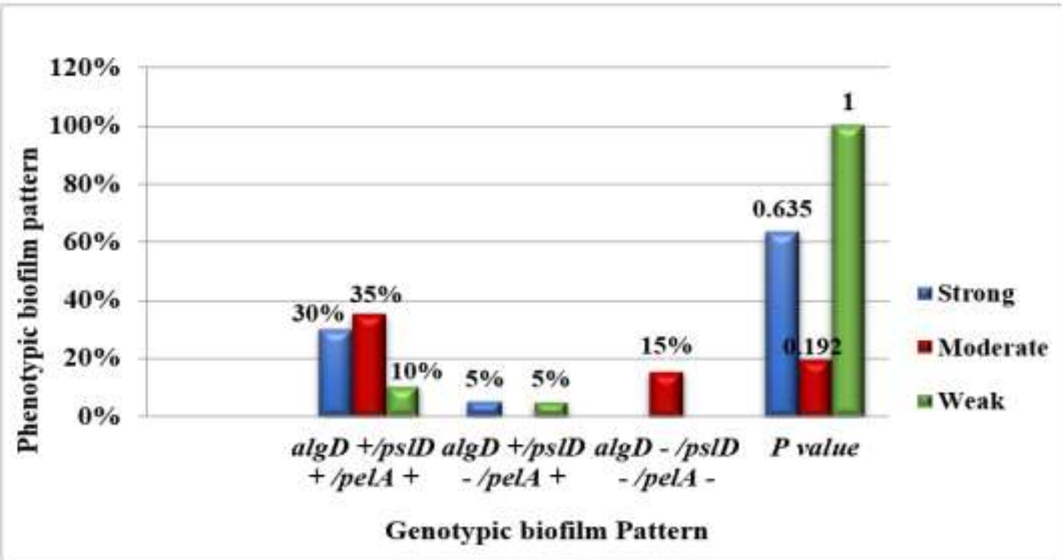


Figure 4.10 Genotype and phenotype biofilm distribution

The results of a genotypic study (p-value 0.764) with chi-square analysis revealed an extremely important link between the production of biofilms and the genesis associated with it, that obtained high correlation between the capacity of strong biofilm and genotype pattern (p value 0.635), besides that the test showed a highly correlation between the capacity of moderate biofilm and genotype pattern (p value 0.192), and high important correlation between genotype pattern and the ability of weak biofilm forming (p value 1).

The results close to other studies by (Alrawi and Mahmood 2022, Kamali *et al.* 2020).

5 CONCLUSIONS AND RECOMMENDATION

The ability of *P. aeruginosa* to easily colonize the host and its mechanism of action through virulence factors and genes made it a critical pathogen and needs care and monitoring before it worsens.

The data we obtained by this research show different clinical isolates from *P. aeruginosa* which have different resistance profiles were detected at Baghdad hospitals. It has been hypothesized that the high levels of antimicrobial resistance by cephalosporin like (cefazolin) and glycylcyclines like (Tigecycline) most likely are due to inappropriate doses that given to patients and that resistance is considerable because it limits the number of reliable antibiotics for treating patient with sever wound burned or lung infection.

There is Multiple factors are involved in the effect of antimicrobial therapies, especially since they may have been given to patients previously, and wrong instruction in using medical apparatus, in addition *P. aeruginosa* is a normal flora in human body and frequent entry into the body may help increase resistance to certain antibiotics through the formation of biofilms by the autoinducer in the controlled quorum sensing system, because the acquisition of resistance induce bacteria to be stronger and that helping bacteria to compete and assist in the colonization process.

We can reduce the resistance against Tigecycline and cefazolin by doing the susceptibility test to make the right choice (dose or concentration) and avoid the long therapy of the same antibiotics without any response from patient.

We discovered that biofilm genes were equally present in a significant proportion of samples and predominantly in respiratory tract infection and wound infection, and were less common in burn infection in addition to biofilm formation, reflecting the active role of biofilm for such injuries and associated genes (*algD*, *pslD* and *pelA*), which revealed the strong correlation between biofilm formation and biofilm related genes, through the crucial role in increasing pathogenicity in these isolates, In respect to the results of the analysis data, it revealed a significant linked between biofilm formation and biofilm genes by presence of high rate between them in clinical samples of *P. aeruginosa*.

We noticed the low positive role of *pslD* gene in wound infection which related to the lack in function of psl in pathogenicity with this type of infection.

With respect, that representing the formation of biofilm mechanism rely on alginate as essential ESP and Pel and psl polysaccharides matrices in both respiratory, wound and burn wound infection, with some modification in prevalence.

It is revealed the obvious involved of biofilm which indicates the prevalent of its related to the pathogenicity particularly in these isolates, besides that we discovered the presence of biofilm regardless of the lack of biofilm genes in some isolates and that explain role of many factors that affect production of biofilm. And all of that highlight on the existence of alternative biofilm genetic factors and their role in matrics development and we realized that other genes may not yet investigated also play a function in biofilm creation.

By Finding all of that, we must discover and use a new approach in impairing the strong bindings between biofilm formation and biofilm genes (stop function of gene or prevent producing biofilm), which may prevent pathogenicity in patients especially with immune comprised system and inspire us to reduce their clinical affect.

That's making us stop at this point: what is the right period for pathogenicity? And how can we discover it before that happens? How to stop the deep invasion of this pathogenic? What is the right method to stop biofilm production and reduce the virulence factors? It can be done by rapid discovery of related virulence genes, right choice of antibiotics and identify main reason of biofilm formation, this can be lighten the way in protecting people health.

Depending on phenotype detection of *algD*, *pelA*, and *pslD* outcomes, we found that most of the local isolates are mucoid and that indicates presence of (alginate) as incidence of *algD* gene in isolates, which explain that biofilm formation process depends on alginate as essential ESP apart from Pel and Psl matrices. So local isolates was found in mucoid pattern to enhance their ability of convert into chronic mode, on the other side that helps in persistent the pathogenicity of isolates via transformation in mucoidity, furthermore the resistance to antibiotic mechanisms to evade organism immunity and antimicrobial agents

Wide research in our country and world should show the variety of resistance of *P. aeruginosa* bacteria profile and the relationship between biofilm development and the capacity of its virulence genes on the intense infection.

Advanced developments can help understanding the activity role of pyocyanin in infection, classifying the proper strains which are timely exhibit the pigment as the infection clinical isolates of *P.aeruginosa* is gaining wide prevalence.

REFERENCES

Abd Al Rhman, E. A., Abd El Haliem, N. F., Hassan, E. M., & Al, L. I. 2018. Phenotypic and Genotypic Characterizations of Staphylococcal Biofilm Formation from Neonatal Infection Isolates. The Egyption Journal of Medical Microbiology, 2: 1-9.

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

Ahmed, I., Rabbi, M. B., & Sultana, S. 2019. Antibiotic resistance in Bangladesh: A systematic review. International Journal of Infectious Diseases, 80: 54-61.

Ahmed, M. M. E. 2021. Polymyxins:“Last Resort” for MDR and/or XDR Gram-Negative Infections. Journal of Scientific Research in Medical and Biological Sciences, 2(3): 123-141.

Al Dawodeyah, H. Y., Obeidat, N., Abu-Qatouseh, L. F., & Shehabi, A. A. 2018. Antimicrobial resistance and putative virulence genes of Pseudomonas aeruginosa isolates from patients with respiratory tract infection. Germs, 8(1): 31.

Al, M. A. A. R. H., Musleh, L. N., & Al-Mathkhury, H. J. 2019. Assessment of pelA-carried Pseudomonas aeruginosa isolates in respect to biofilm formation. Iraqi Journal of Science, 1180-1187.

Albelda-Berenguer, M., Monachon, M., & Joseph, E. 2019. Siderophores: From natural roles to potential applications. Advances in applied microbiology, 106: 193-225.

Al-Fridawy, R. A. K., Al-Daraghi, W. A. H., & Alkhafaji, M. H. 2020. Isolation and Identification of Multidrug Resistance Among Clinical and Environmental Pseudomonas aeruginosa Isolates. Iraqi journal of biotechnology, 19(2).

Ali, A. M., Al-Kenanei, K. A., & Bdaiwi, Q. O. 2020. Molecular study of some virulence genes of Pseudomonas aeruginosa isolated from different infections in hospitals of Baghdad. Reviews in Medical Microbiology, 31(1): 26-41.

Alkhudhairy, M. K., & Al-Shammari, M. M. M. 2020. Prevalence of metallo-β-lactamase–producing Pseudomonas aeruginosa isolated from diabetic foot infections in Iraq. New microbes and new infections, 35: 100661.

Allegretta, G., Maurer, C. K., Eberhard, J., Maura, D., Hartmann, R. W., Rahme, L., & Empting, M. 2017. In-depth profiling of MvfR-regulated small molecules in Pseudomonas aeruginosa after quorum sensing inhibitor treatment. Frontiers in microbiology, 8: 924.

Alrawi, D. K., & Mahmood, H. M. 2022. Prevalence of Biofilm Genotype Pattern (algD−/pslD−/pelF–) with Multidrug-Resistant in Clinical Local Pseudomonas Aeruginosa Isolates. Indian Journal of Forensic Medicine & Toxicology, 16(1): 381.

Al-Wrafy, F., Brzozowska, E., Górska, S., & Gamian, A. 2017. Pathogenic factors of Pseudomonas aeruginosa-the role of biofilm in pathogenicity and as a target for phage therapy. Advances in Hygiene & Experimental Medicine/Postepy Higieny i Medycyny Doswiadczalnej, 71.

Arsovic, N., Radivojevic, N., Jesic, S., Babac, S., Cvorovic, L., & Dudvarski, Z. 2020. Malignant otitis externa: causes for various treatment responses. The Journal of International Advanced Otology, 16(1): 98.

Arumugham, V. B., Gujarathi, R., & Cascella, M. 2019. Third generation cephalosporins.

Asadpour, L. 2018. Antimicrobial resistance, biofilm-forming ability and virulence potential of Pseudomonas aeruginosa isolated from burn patients in northern Iran. Journal of global antimicrobial resistance, 13: 214-220.

Atshan, S. S., Nor Shamsudin, M., Sekawi, Z., Lung, L. T. T., Hamat, R. A., Karunanidhi, A., & Pei Pei, C. 2012. Prevalence of adhesion and regulation of biofilm-related genes in different clones of Staphylococcus aureus. Journal of BioMedicine and biotechnology, 2012.

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

Bassetti, M., Carnelutti, A., & Peghin, M. 2017. Patient specific risk stratification for antimicrobial resistance and possible treatment strategies in gram-negative bacterial infections. Expert review of anti-infective therapy, 15(1): 55-65.

Bassetti, M., Vena, A., Croxatto, A., Righi, E., & Guery, B. 2018. How to manage Pseudomonas aeruginosa infections. Drugs in context, 7.

Becker, B., & Cooper, M. A. 2013. Aminoglycoside antibiotics in the 21st century. ACS chemical biology, 8(1): 105-115.

Bédard, E., Prévost, M., & Déziel, E. 2016. Pseudomonas aeruginosa in premise plumbing of large buildings. Microbiologyopen, 5(6): 937-956.

Behzadi, P., Baráth, Z., & Gajdács, M. 2021. It's not easy being green: a narrative review on the microbiology, virulence and therapeutic prospects of multidrug-resistant Pseudomonas aeruginosa. Antibiotics, 10(1): 42.

Belaynehe, K. M., Shin, S. W., Hong-Tae, P., & Yoo, H. S. 2017. Occurrence of aminoglycoside-modifying enzymes among isolates of Escherichia coli exhibiting high levels of aminoglycoside resistance isolated from Korean cattle farms. FEMS Microbiology Letters, 364(14).

Bengtsson-Palme, J., Kristiansson, E., & Larsson, D. J. 2018. Environmental factors influencing the development and spread of antibiotic resistance. FEMS microbiology reviews, 42(1): fux053.

Billings, N., Ramirez Millan, M., Caldara, M., Rusconi, R., Tarasova, Y., Stocker, R., & Ribbeck, K. 2013. The extracellular matrix component Psl provides fast-acting antibiotic defense in Pseudomonas aeruginosa biofilms. PLoS pathogens, 9(8): e1003526.

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.

Blankenfeldt, W., & Parsons, J. F. 2014. The structural biology of phenazine biosynthesis. Current opinion in structural biology, 29: 26-33.

Brauner, A., Fridman, O., Gefen, O., & Balaban, N. Q. 2016. Distinguishing between resistance, tolerance and persistence to antibiotic treatment. Nature Reviews Microbiology, 14(5): 320-330.

Bush, K. 2018. Past and present perspectives on β-lactamases. Antimicrobial agents and chemotherapy, 62(10): e01076-18.

Bush, K., & Bradford, P. A. 2016. β-Lactams and β-lactamase inhibitors: an overview. Cold Spring Harbor perspectives in medicine, 6(8): a025247.

Cartotto, R. 2017. Topical antimicrobial agents for pediatric burns. Burns & trauma, 5.

Cavalier-Smith, T. 2004. Only six kingdoms of life. Proceedings of the Royal Society of London. Series B: Biological Sciences, 271(1545): 1251-1262.

Christiaen, S. E., Matthijs, N., Zhang, X. H., Nelis, H. J., Bossier, P., & Coenye, T. 2014. Bacteria that inhibit quorum sensing decrease biofilm formation and virulence in Pseudomonas aeruginosa PAO1. Pathogens and disease, 70(3): 271-279.

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, 10: 913.

Colvin, K. M., Alnabelseya, N., Baker, P., Whitney, J. C., Howell, P. L., & Parsek, M. R. 2013. PelA deacetylase activity is required for Pel polysaccharide synthesis in Pseudomonas aeruginosa. Journal of bacteriology, 195(10): 2329-2339.

Costa, T. R., Felisberto-Rodrigues, C., Meir, A., Prevost, M. S., Redzej, A., Trokter, M., & Waksman, G. 2015. Secretion systems in Gram-negative bacteria: structural and mechanistic insights. Nature Reviews Microbiology, 13(6): 343-359.

Cui, C. Y., He, Q., Jia, Q. L., Li, C., Chen, C., Wu, X. T., & Sun, J. 2021. Evolutionary trajectory of the Tet (X) family: critical residue changes towards high-level tigecycline resistance. Msystems, 6(3): e00050-21.

Cunrath, O., Graulier, G., Carballido-Lopez, A., Pérard, J., Forster, A., Geoffroy, V. A., & Fechter, P. 2020. The pathogen Pseudomonas aeruginosa optimizes the production of the siderophore pyochelin upon environmental challenges. Metallomics, 12(12): 2108-2120.

Das, T., & Manefield, M. 2012. Pyocyanin promotes extracellular DNA release in Pseudomonas aeruginosa.

Das, T., Manoharan, A., Whiteley, G., Glasbey, T., & Manos, J. 2020. Pseudomonas aeruginosa biofilms and infections: Roles of extracellular molecules. In New and future developments in microbial biotechnology and bioengineering: microbial biofilms (pp. 29-46). Elsevier.

David, S. M., Jayaprakash, C., & Mathew, A. 2020. Isolation, Identification and Antibiotic Susceptibility Testing of Pseudomonas aeruginosa and Acinetobacter baumannii from Endotracheal Secretions in a Tertiary Care Centre. Int. J. Curr. Microbiol. App. Sci, 9(2): 1566-1574.

Diggle, S. P., & Whiteley, M. 2020. Microbe Profile: Pseudomonas aeruginosa: opportunistic pathogen and lab rat. Microbiology, 166(1): 30.

Ding, J., Gao, X., Gui, H., Ding, X., Lu, Y., An, S., & Liu, Q. 2021. Proteomic Analysis of Proteins Associated with Inhibition of Pseudomonas aeruginosa Resistance to Imipenem Mediated by the Chinese Herbal Medicine Qi Gui Yin. Microbial Drug Resistance, 27(4): 462-470.

Divyashree, M., Mani, M. K., & Karunasagar, I. 2022. Association of exopolysaccharide genes in biofilm developing antibiotic-resistant Pseudomona s aeruginosa from hospital wastewater. Journal of Water and Health, 20(1): 176-184.

Durand, M. L. 2013. Endophthalmitis. Clinical Microbiology and Infection, 19(3): pp.227-234.

Elmaraghy, N., Abbadi, S., Elhadidi, G., Hashem, A., & Yousef, A. 2019. Virulence genes in Pseudomonas aeruginosa strains isolated at Suez Canal University Hospitals with respect to the site of infection and antimicrobial resistance. International Journal of Clinical Microbiology and Biochemical Technology, 2(1): 008-019.

f, F., Paolucci, I. A., Ceccarelli, M., d'Aleo, F., Iannello, D., Facciolà, A., & Pellicanò, G. F. 2018. Pseudomonas aeruginosa detection in South Italy: an epidemiological survey. Infect Dis Trop Med, 4(4): e507.

Faiz, M. A., Ding, C. H., Wahab, A. A., Tzar, M. N., Sulong, A., Wong, K. K., & Wong, P. F. 2022. The Antibiotic Resistance Profile of Pseudomonas Aeruginosa in a Tertiary Medical Center from Malaysia. Journal of Medicine, 23(1): 54-60.

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.

Faure, E., Kwong, K., & Nguyen, D. 2018. Pseudomonas aeruginosa in chronic lung infections: how to adapt within the host?. Frontiers in immunology, 9: 2416.

Fila, G., Krychowiak, M., Rychlowski, M., Bielawski, K. P., & Grinholc, M. 2018. Antimicrobial blue light photoinactivation of Pseudomonas aeruginosa: Quorum sensing signaling molecules, biofilm formation and pathogenicity. Journal of biophotonics, 11(11): e201800079.

Fujii, A., Seki, M., Higashiguchi, M., Tachibana, I., Kumanogoh, A., & Tomono, K. 2014. Community-acquired, hospital-acquired, and healthcare-associated pneumonia caused by Pseudomonas aeruginosa. Respiratory medicine case reports, 12: 30-33.

Fujitani, S., Moffett, K. S., & Yu, V. L. 2017. Pseudomonas aeruginosa. Antimicrobe, Pittsburgh, PA, 15219.

Gagliardo, C., & Saiman, L. 2015. Microbiology of cystic fibrosis: Epidemiology of cystic fibrosis pathogens and clinical microbiology laboratory methods. Hodson and Geddes' Cystic Fibrosis., 174.

Gallagher, J. C., & MacDougall, C. 2022. Antibiotics simplified. Jones & Bartlett Learning.

Ghadaksaz, A., Fooladi, A. A. I., Hosseini, H. M., & Amin, M. 2015. The prevalence of some Pseudomonas virulence genes related to biofilm formation and alginate production among clinical isolates. Journal of Applied Biomedicine, 13(1): 61-68.

Ghafoor, A., Jordens, Z., & Rehm, B. H. 2013. Role of PelF in Pel polysaccharide biosynthesis in Pseudomonas aeruginosa. Applied and environmental microbiology, 79(9): 2968-2978.

Gloag, E. S., Turnbull, L., Huang, A., Vallotton, P., Wang, H., Nolan, L. M., & Whitchurch, C. B. 2013. Self-organization of bacterial biofilms is facilitated by extracellular DNA. Proceedings of the National Academy of Sciences, 110(28): 11541-11546.

Gonçalves, T., & Vasconcelos, U. 2021. Colour me blue: the history and the biotechnological potential of pyocyanin. Molecules, 26(4): 927.

Gonzalez, M. R., Fleuchot, B., Lauciello, L., Jafari, P., Applegate, L. A., Raffoul, W., & Perron, K. 2016. Effect of human burn wound exudate on Pseudomonas aeruginosa virulence. Msphere, 1(2): e00111-15.

Hansen, B. A., Wendelbo, Ø., Bruserud, Ø., Hemsing, A. L., Mosevoll, K. A., & Reikvam, H. 2020. Febrile neutropenia in acute leukemia. Epidemiology, etiology, pathophysiology and treatment. Mediterranean journal of hematology and infectious diseases, 12(1).

Holban, A. M., Chifiriuc, M. C., Cotar, A. I., Bleotu, C., Grumezescu, A. M., Banu, O., & Lazar, V. 2013. Virulence markers in Pseudomonas aeruginosa isolates from hospital acquired infections occurred in patients with underlying cardiovascular disease. Romanian Biotechnological Letters, 18(6): 8843-8854.

Horcajada, J. P., Montero, M., Oliver, A., Sorlí, L., Luque, S., Gómez-Zorrilla, S., & Grau, S. 2019. Epidemiology and treatment of multidrug-resistant and extensively drug-resistant Pseudomonas aeruginosa infections. Clinical microbiology reviews, 32(4): e00031-19.

Huszczynski, S. M., Lam, J. S., & Khursigara, C. M. 2019. The role of Pseudomonas aeruginosa lipopolysaccharide in bacterial pathogenesis and physiology. Pathogens, 9(1): 6.

Idowu, T., Ammeter, D., Brizuela, M., Jackson, G., Alam, S., & Schweizer, F. 2020. Overcoming β-Lactam resistance in Pseudomonas aeruginosa using non-canonical tobramycin-based antibiotic adjuvants. Bioorganic & Medicinal Chemistry Letters, 30(21): 127575.

Isichei-Ukah, B. O., & Enabulele, O. I. 2020. Analysis of Class 1 Integrons and Antibiotic Resistance Genes in Pseudomonas aeruginosa Strains from Benin City, Nigeria. Journal of Applied Sciences and Environmental Management, 24(4): 633-637.

Ismail, S. J., & Mahmoud, S. S. 2018. First detection of New Delhi metallo-β-lactamases variants (NDM-1, NDM-2) among Pseudomonas aeruginosa isolated from Iraqi hospitals. Iranian journal of microbiology, 10(2): 98.

Jacob, J. S., & Tschen, J. 2020. Hot tub-associated Pseudomonas folliculitis: a case report and review of host risk factors. Cureus, 12(9).

Jones, C. J., & Wozniak, D. J. 2017. Psl produced by mucoid Pseudomonas aeruginosa contributes to the establishment of biofilms and immune evasion. MBio, 8(3): e00864-17.

Kadum, M. M., AL-Marjani, M. F., & Ali, M. R. 2019. Colony Morphology and pelA, pslD and algD genes in Pseudomonas aeruginosa isolated from Baghdad Hospitals.

Kafil, H. S., Mobarez, A. M., Moghadam, M. F., sadat Hashemi, Z., & Yousefi, M. 2016. Gentamicin induces efaA expression and biofilm formation in Enterococcus faecalis. Microbial pathogenesis, 92: 30-35.

Kalil, A. C., Metersky, M. L., Klompas, M., Muscedere, J., Sweeney, D. A., Palmer, L. B., & Brozek, J. L. 2016. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clinical Infectious Diseases, 63(5): e61-e111.

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.

Karballaei Mirzahosseini, H., Hadadi-Fishani, M., Morshedi, K., & Khaledi, A. 2020. Meta-analysis of biofilm formation, antibiotic resistance pattern, and biofilm-related genes in Pseudomonas aeruginosa isolated from clinical samples. Microbial Drug Resistance, 26(7): 815-824.

Karbasizade, V., Heidari, L., & Jafari, R. 2015. Detection of oxa-type carbapenemase genes in Acinetobacter baumannii isolates from nosocomial infections in Isfahan hospitals, Iran. Journal of Medical Bacteriology, 4(5-6): 31-36.

Kathleen, P. T. 2017. Foundations in microbiology: basic principles. Mcgraw-Hill US Higher Ed.

Kayser, F. H., Bienz, K. A., Eckert, J., & Zinkernagel, R. M. 2005. Medical microbiology.

Kempf, M., & Rolain, J. M. 2012. Emergence of resistance to carbapenems in Acinetobacter baumannii in Europe: clinical impact and therapeutic options. International journal of antimicrobial agents, 39(2): 105-114.

Keyal, N. K., Shrestha, M., & Ghimire, P. S. 2020. Antimicrobial Sensitivity Pattern in the Mixed Intensive Care Unit in a Tertiary Care Hospital of Eastern Nepal. Eastern Green Neurosurgery, 2(2): 9-15.

Khan, M., Summers, S., Rice, S. A., Stapleton, F., Willcox, M. D., & Subedi, D. 2020. Acquired fluoroquinolone resistance genes in corneal isolates of Pseudomonas aeruginosa. Infection, Genetics and Evolution, 85: 104574.

Kim, J. S., Yamasaki, R., Song, S., Zhang, W., & Wood, T. K. 2018. Single cell observations show persister cells wake based on ribosome content. Environmental microbiology, 20(6): 2085-2098.

Lamas Ferreiro, J. L., Álvarez Otero, J., González González, L., Novoa Lamazares, L., Arca Blanco, A., Bermúdez Sanjurjo, J. R., & de la Fuente Aguado, J. 2017. Pseudomonas aeruginosa urinary tract infections in hospitalized patients: Mortality and prognostic factors. PloS one, 12(5): e0178178.

Lari, A. R., Azimi, L., Soroush, S., & Taherikalani, M. 2015. Low prevalence of metallo-beta-lactamase in Pseudomonas aeruginosa isolated from a tertiary burn care center in Tehran. International journal of immunopathology and pharmacology, 28(3): 384-389.

Laverty, G., Gorman, S. P., & Gilmore, B. F. 2014. Biomolecular mechanisms of Pseudomonas aeruginosa and Escherichia coli biofilm formation. Pathogens, 3(3): 596-632.

Lebeaux, D., Ghigo, J. M., & Beloin, C. 2014. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiology and Molecular Biology Reviews, 78(3): 510-543.

Lee, J., & Zhang, L. 2015. The hierarchy quorum sensing network in Pseudomonas aeruginosa. Protein & cell, 6(1): 26-41.

Lee, J., Wu, J., Deng, Y., Wang, J., Wang, C., Wang, J., & Zhang, L. H. 2013. A cell-cell communication signal integrates quorum sensing and stress response. Nature chemical biology, 9(5): 339-343.

Levinson, W. 2014. Review of medical microbiology and immunology. McGraw-Hill Education.

Lin, J., & Cheng, J. 2019. Quorum sensing in pseudomonas aeruginosa and its relationship to biofilm development. In Introduction to Biofilm Engineering (pp. 1-16). American Chemical Society.

Liu, D., Zhai, W., Song, H., Fu, Y., Schwarz, S., He, T., & Shen, J. 2020. Identification of the novel tigecycline resistance gene tet (X6) and its variants in Myroides, Acinetobacter and Proteus of food animal origin. Journal of Antimicrobial Chemotherapy, 75(6): 1428-1431.

Long, S. S., Prober, C. G., & Fischer, M. 2022. Principles and practice of pediatric infectious diseases E-Book. Elsevier Health Sciences.

López-Causapé, C., Cabot, G., del Barrio-Tofiño, E., & Oliver, A. 2018. The versatile mutational resistome of Pseudomonas aeruginosa. Frontiers in microbiology, 9: 685.

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

Mahajan, S., Sunsunwal, S., Gautam, V., Singh, M., & Ramya, T. N. C. 2021. Biofilm inhibitory effect of alginate lyases on mucoid P. aeruginosa from a cystic fibrosis patient. Biochemistry and Biophysics Reports, 26: 101028.

Marko, D. C., Saffert, R. T., Cunningham, S. A., Hyman, J., Walsh, J., Arbefeville, S., ... & Richter, S. S. 2012. Evaluation of the Bruker Biotyper and Vitek MS matrix-assisted laser desorption ionization–time of flight mass spectrometry systems for identification of nonfermenting Gram-negative bacilli isolated from cultures from cystic fibrosis patients. Journal of clinical microbiology, 50(6): 2034-2039.

Marmont, L. S., Whitfield, G. B., Rich, J. D., Yip, P., Giesbrecht, L. B., Stremick, C. A., & Howell, P. L. 2017. PelA and PelB proteins form a modification and secretion complex essential for Pel polysaccharide-dependent biofilm formation in Pseudomonas aeruginosa. Journal of Biological Chemistry, 292(47): 19411-19422.

Marrez, D. A., & Mohamad, H. S. 2020. Biological activity and applications of pyocyanin produced by Pseudomonas aeruginosa.

Maurice, N. M., Bedi, B., & Sadikot, R. T. 2018. Pseudomonas aeruginosa biofilms: host response and clinical implications in lung infections. American journal of respiratory cell and molecular biology, 58(4): 428-439.

Millar, B. C., McCaughan, J., Rendall, J. C., & Moore, J. E. 2021. Delafloxacin—A novel fluoroquinolone for the treatment of ciprofloxacin- resistant Pseudomonas aeruginosa in patients with cystic fibrosis. The Clinical Respiratory Journal, 15(1): 116-120.

Moehario, L. H., Boestami, H. P., Edbert, D., Tjoa, E., & Robertus, T. 2019. Automation for the identification of Pseudomonas aeruginosa: Comparison of TDR-300B, VITEK® 2 and VITEK®-MS. bioRxiv, 510107.

Moghaddam, M. M., Khodi, S., & Mirhosseini, A. 2014. Quorum Sensing in Bacteria and a Glance on Pseudomonas aeruginosa. Clin Microbial, 3(4): 156.

Moissenet, D., & Khedher, M. 2011. Virulence factors in Pseudomonas aeruginosa: mechanisms and modes of regulation. In Annales de biologie clinique (Vol. 69, No. 4, pp. 393-403).

Molla, M. R., Haji, H. K., & Molla, N. M. 2020. View from the Other Side: A Perspective on Oral and Maxillofacial Surgery in a Developing Nation-Bangladesh. Oral and Maxillofacial Surgery Clinics, 32(3): 377-388.

Moore, N. M., & Flaws, M. L. 2011. Epidemiology and pathogenesis of Pseudomonas aeruginosa infections. Clinical laboratory science, 24(1): 43.

Moradali, M. F., Ghods, S., & Rehm, B. H. 2017. Activation mechanism and cellular localization of membrane-anchored alginate polymerase in Pseudomonas aeruginosa. Applied and environmental microbiology, 83(9): e03499-16.

Moradali, M. F., Ghods, S., & Rehm, B. H. 2017. Pseudomonas aeruginosa lifestyle: a paradigm for adaptation, survival, and persistence. Frontiers in cellular and infection microbiology, 7: 39.

Ms, A., A Salim, A. T. E. F., & Mf, E. D. 2021. Virulence of isolated pseudomonas aeruginosa infecting duckling and antibiotic resıstance with an experimental treatment trial. Assiut Veterinary Medical Journal, 67(169): 74-90.

Mubarak, K. I. 2021. Detection of Some Virulence Factors and Antibiotics Susceptibility of Psudomonas aeruginosa Clinical Isolates.

Murray, P. R., & Shea, Y. R. 2004. Pocket guide to clinical microbiology. Washington, DC: Asm Press.

Ochs, M. A., Dillman, N. O., Caverly, L. J., & Chaffee, V. D. 2021. Aminoglycoside dosing and monitoring for Pseudomonas aeruginosa during acute pulmonary exacerbations in cystic fibrosis. Pediatric Pulmonology, 56(12): 3634-3643.

Odumosu, B. T., Adeniyi, B. A., & Chandra, R. 2015. Occurrence of aminoglycoside-modifying enzymes genes (aac (6′)-I and ant (2″)-I) in clinical isolates of Pseudomonas aeruginosa from Southwest Nigeria. African health sciences, 15(4): 1277-1281.

Ohn, J., Yu, D. A., Park, H., Cho, S., & Mun, J. H. 2020. Green nail syndrome: analysis of the association with onychomycosis. Journal of the American Academy of Dermatology, 83(3): 940-942.

Olivares, E., Badel-Berchoux, S., Provot, C., Prévost, G., Bernardi, T., & Jehl, F. 2020. Clinical impact of antibiotics for the treatment of Pseudomonas aeruginosa biofilm infections. Frontiers in microbiology, 10: 2894.

Oumeri, M. M. Q., & Yassin, N. A. 2021. Molecular characterizatıon of some carbapenem-resistance genes among pseudomonas aeruginosa isolated from wound and burn infections ın Duhok Cıty, Iraq. Journal of Duhok University, 24(1): 136-144.

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

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.

Patel, J. B., Cockerill, F. R. and Bradford, P. A. 2015. Performance standards for antimicrobial susceptibility testing: twenty-fifth informational supplement.

Perez, L. R. R., Freitas, A. L. P. D., & Barth, A. L. 2011. Nutritional requirement among Pseudomonas aeruginosa isolates recovered from respiratory clinical specimens at a tertiary hospital from South of Brazil. Brazilian Journal of Microbiology, 42: 1061-1064.

Prabhurajeshwar, C. 2019. A Review on Emergence of Antibiotic Resistance in Pseudomonas aeruginosa; Its Risk Factors and Clinical Impacts. International Journal of Recent Scientific Research, 10.

Prince, A. S. 2012. Pseudomonas aeruginosa. Principles and Practice of Pediatric Infectious Diseases, pp.842-846.

Pulcrano, G., Iula, D. V., Raia, V., Rossano, F., & Catania, M. R. 2012. Different mutations in mucA gene of Pseudomonas aeruginosa mucoid strains in cystic fibrosis patients and their effect on algU gene expression. New Microbiologica, 35(3): 295-305.

Rada, B., Jendrysik, M. A., Pang, L., Hayes, C. P., Yoo, D. G., Park, J. J., & Leto, T. L. 2013. Pyocyanin-enhanced neutrophil extracellular trap formation requires the NADPH oxidase. PloS one, 8(1): e54205.

Ramakrishnan, M., Bai, S. P., & Babu, M. 2016. Study on biofilm formation in burn wound infection in a pediatric hospital in Chennai, India. Annals of burns and fire disasters, 29(4): 276.

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

Riera, E., Cabot, G., Mulet, X., García-Castillo, M., del Campo, R., Juan, C., & Oliver, A. 2011. Pseudomonas aeruginosa carbapenem resistance mechanisms in Spain: impact on the activity of imipenem, meropenem and doripenem. Journal of antimicrobial chemotherapy, 66(9): 2022-2027.

Rooku, K. C., Adhikari, S., Bastola, A., Devkota, L., Bhandari, P., Ghimire, P., & Ghimire, P. 2019. Opportunistic respiratory infections in HIV patients attending sukraraj tropical and infectious diseases hospital in Kathmandu, Nepal. HIV/AIDS (Auckland, NZ), 11: 357.

Sahu, M. C., Swain, S. K., & Kar, S. K. 2019. Genetically diversity of Pseudomonas aeruginosa isolated from chronic suppurative otitis media with respect to their antibiotic sensitivity pattern. Indian Journal of Otolaryngology and Head & Neck Surgery, 71(2): 1300-1308.

Saipriya, K., Swathi, C. H., Ratnakar, K. S., & Sritharan, V. 2020. Quorum- sensing system in Acinetobacter baumannii: a potential target for new drug development. Journal of applied microbiology, 128(1): 15-27.

Sambrook, J., Fritsch, E. F., & Maniatis, T. 1989. Molecular cloning: a laboratory manual (No. Ed. 2). Cold spring harbor laboratory press.

Sarkis-Ivanova, V. 2017. The formation of biofilms by Pseudomonas aeruginosa strains, and methods of its control. Annals of Mechnikov's Institute, (1): 9-13.

Shah, N., Klaponski, N., Selin, C., Rudney, R., Fernando, W. D., Belmonte, M. F., & De Kievit, T. R. 2016. PtrA is functionally intertwined with GacS in regulating the biocontrol activity of Pseudomonas chlororaphis PA23. Frontiers in microbiology, 7: 1512.

Spagnolo, A. M., Orlando, P., Perdelli, F., & Cristina, M. L. 2016. Hospital water and prevention of waterborne infections. Reviews and Research in Medical Microbiology, 27(1): 25-32.

Spagnolo, A. M., Sartini, M., & Cristina, M. L. 2021. Pseudomonas aeruginosa in the healthcare facility setting. Reviews in Medical Microbiology, 32(3): 169-175.

Streeter, K., & Katouli, M. 2016. Pseudomonas aeruginosa: a review of their pathogenesis and prevalence in clinical settings and the environment.

Strempel, N., Neidig, A., Nusser, M., Geffers, R., Vieillard, J., Lesouhaitier, O., & Overhage, J. 2013. Human host defense peptide LL-37 stimulates virulence factor production and adaptive resistance in Pseudomonas aeruginosa. PloS one, 8(12): e82240.

Subedi, D., Vijay, A. K., & Willcox, M. 2018. Overview of mechanisms of antibiotic resistance in Pseudomonas aeruginosa: an ocular perspective. Clinical and Experimental Optometry, 101(2): 162-171.

Szymańska, M., Karakulska, J., Sobolewski, P., Kowalska, U., Grygorcewicz, B., Böttcher, D., ... & Drozd, R. 2020. Glycoside hydrolase (PelAh) immobilization prevents Pseudomonas aeruginosa biofilm formation on cellulose-based wound dressing. Carbohydrate polymers, 246: 116625.

Tan, J., Rouse, S. L., Li, D., Pye, V. E., Vogeley, L., Brinth, A. R., & Caffrey, M. 2014. A conformational landscape for alginate secretion across the outer membrane of Pseudomonas aeruginosa. Acta Crystallographica Section D: Biological Crystallography, 70(8): 2054-2068.

Tang, Y. W., & Sails, A. 2014. Molecular medical microbiology. Academic press.

Tariq, M., Ansari, F., Rafiq, M. U., Azhar, A., Arshad, F., & Nayab, A. 2020. Evaluation of Multi-Drug Resistance in Biofilm Forming Pseudomonas Aeruginosa Isolated from Urinary Catheter Tips in Patients from ICU and CCU in DHQ Teaching Hospital Gujranwala.

Thi, M. T. T., Wibowo, D., & Rehm, B. H. 2020. Pseudomonas aeruginosa biofilms. International journal of molecular sciences, 21(22): 8671.

Thirumalmuthu, K., Devarajan, B., Prajna, L., & Mohankumar, V. 2019. Mechanisms of fluoroquinolone and aminoglycoside resistance in Keratitis-Associated Pseudomonas aeruginosa. Microbial Drug Resistance, 25(6): 813-823.

Tille, P. 2017. Bailey & Scott's diagnostic microbiology-E-Book. Elsevier Health Sciences.

Turnbull, L., Toyofuku, M., Hynen, A. L., Kurosawa, M., Pessi, G., Petty, N. K., & Whitchurch, C. B. 2016. Explosive cell lysis as a mechanism for the biogenesis of bacterial membrane vesicles and biofilms. Nature communications, 7(1): 1-13.

Vickers, N. J. 2017. Animal communication: when i'm calling you, will you answer too?. Current biology, 27(14): R713-R715.

Wallace, W. S. D. S. M., Beshear, S., Williams, D., Hospadar, S., & Owens, M. 1998. Perchlorate reduction by a mixed culture in an up-flow anaerobic fixed bed reactor. Journal of Industrial Microbiology and Biotechnology, 20(2): 126-131.

Wei, Q., & Ma, L. Z. 2013. Biofilm matrix and its regulation in Pseudomonas aeruginosa. International journal of molecular sciences, 14(10): 20983-21005.

Willyard, C. 2017. Drug-resistant bacteria ranked. Nature, 543(7643): 15.

Wood, T. L., Gong, T., Zhu, L., Miller, J., Miller, D. S., Yin, B., & Wood, T. K. 2018. Rhamnolipids from Pseudomonas aeruginosa disperse the biofilms of sulfate-reducing bacteria. NPJ biofilms and microbiomes, 4(1): 1-8.

Wu, M., & Li, X. 2015. Klebsiella pneumoniae and Pseudomonas aeruginosa. In Molecular medical microbiology (pp. 1547-1564). Academic Press.

Wu, W., Jin, Y., Bai, F., & Jin, S. 2015. Pseudomonas aeruginosa. In Molecular medical microbiology (pp. 753-767). Academic Press.

Yang, S., Cheng, X., Jin, Z., Xia, A., Ni, L., Zhang, R. and Jin, F. 2018. Differential production of Psl in planktonic cells leads to two distinctive attachment phenotypes in Pseudomonas aeruginosa. Applied and environmental microbiology, 84(14): pp.e00700-18.

Yin, W., Wang, Y., Liu, L., & He, J. 2019. Biofilms: the microbial "protective clothing" in extreme environments. International journal of molecular sciences, 20(14): 3423.

Zarate, M., Barrantes, D., Cuicapuza, D., Velasquez, J., Fernández, N., Salvatierra, G., & Tamariz, J. 2021. Frequency of colistin resistance in Pseudomonas aeruginosa: first report from Peru. Revista Peruana de Medicina Experimental y Salud Pública, 38: 308-312.

APPENDICES

APPENDIX 1. Antibiotic results by VITEK®2 compact system

APPENDIX 2. Biochemical tests array of VITEK®2 GN cassette (64 tests)

APPENDIX 3. Data of clinical isolates

APPENDIX 4. Biofilm intensity between clinical isolates

APPENDIX 5. Distribution of biofilm genes (*algD*, *pslD* and *pelA*) among clinical isolates

APPENDIX 6. Microtiter plate with ethanol

APPENDIX 7. Stainless Steel laboratory thermostat incubator DH3600BII

APPENDIX 8. Automatic ELISA reader PKL PPC 230

APPENDIX 9. Multichannel micropipette

APPENDIX 10. VITEK 2 complex system

APPENDIX 11. AST (ANTIBIOTIC SUSCEPTIBILITY TEST CARDS)

APPENDIX 12. *Pseudomonas aeruginosa* sub culture on mackonkey agar media

APPENDIX 13. Pyocyanin pigment phenotype

APPENDIX 1. Antibiotic results by VITEK®2 compact system

bioMérieux Customer:

Wahj AL-DNA
Microbiology Chart Report

Printed February 12, 2022 11:07:42 AM CST

Patient Name:
Location:
Lab ID: 8 noor riadh

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Pseudomonas aeruginosa

Source:

Collected:

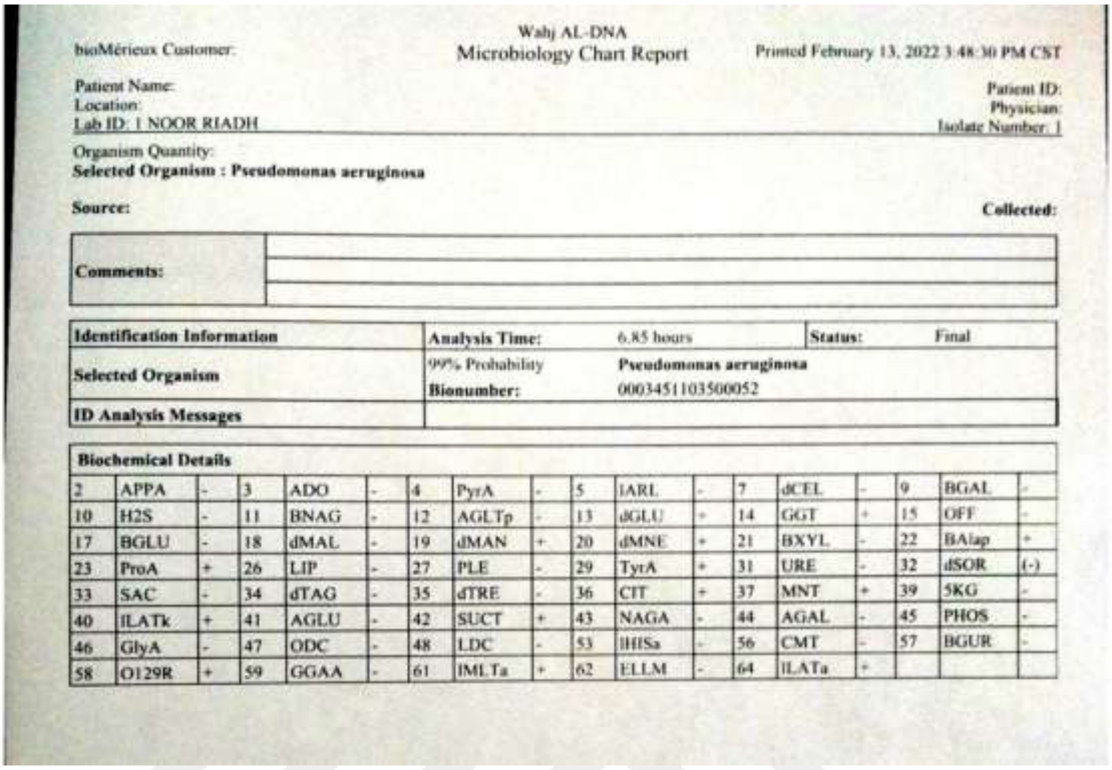
Comments:	

Susceptibility Information	**Analysis Time:** 14.22 hours	**Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Piperacillin/Tazobactam	8	S	Amikacin	<= 2	S
Cefazolin	>= 64	R	Gentamicin	<= 1	S
Ceftazidime	2	S	Ciprofloxacin	<= 0.25	S
Cefepime	<= 1	S	Levofloxacin	0.5	S
Imipenem	2	S	Tigecycline	>= 8	R

AES Findings	
Confidence:	Consistent

APPENDIX 2. Biochemical tests array of VITEK®2 GN cassette (64 tests)



bioMérieux Customer:

Wahj AL-DNA
Microbiology Chart Report

Printed February 13, 2022 3:48:30 PM CST

Patient Name:
Location:
Lab ID: 1 NOOR RIADH

Patient ID:
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Pseudomonas aeruginosa

Source:

Collected:

Comments:	

Identification Information	**Analysis Time:**	6.85 hours	**Status:**	Final
Selected Organism	99% Probability **Bionumber:**	**Pseudomonas aeruginosa** 0003451103500052		
ID Analysis Messages				

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	+			

APPENDIX 3. Data of clinical isolates

Lab No.		Sample type	Age	GENDER	Blood type	Injury period	ESR	CRP
1		Wound	30	Male	O+	3 month	47	26
2		Burn	52	Female	A+	2 month	49	19
3		Sputum	46	Male	AB+	2 month	41	25
4		Wound	40	Male	B-	3 month	35	35
5		Burn	55	Male	O+	2 month	51	16
6		Sputum	48	Female	O+	5 month	37	28
7		Wound	35	Female	O+	2 month	56	32
8		Burn	49	Male	AB+	One month	55	11
9		Burn	58	Male	A+	2 month	41	16
10		Sputum	49	Male	B+	2 month	49	19
11		Wound	51	Male	AB+	3 month	38	36
12		Wound	45	Male	A-	4 month	42	37
13		Sputum	46	Female	O+	2 month	37	25
14		Burn	61	Female	AB+	5 month	43	38
15		Sputum	40	Male	B+	2 month	39	34
16		Sputum	71	Female	O-	4 month	54	19
17		Wound	51	Male	O+	4 month	52	24
18		Burn	44	Female	A+	3 month	57	26
19		Wound	48	Female	AB+	5 month	48	38
20		Burn	33	Male	o+	2 month	46	43

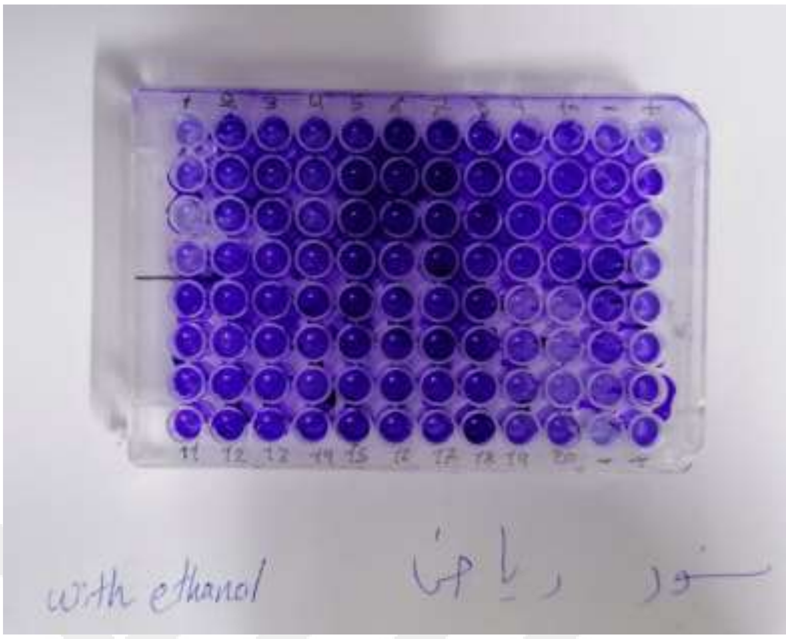
APPENDIX 4. Biofilm intensity between clinical isolates

Lab No.	sample type	DNA Result	PCR *algD* 593bp	PCR *ps1D* 369bp	PCR *Pe1A* 786bp
1	Wound	+	+	-	+
2	Burn	+	+	+	+
3	Sputum	+	+	+	+
4	Wound	+	-	-	-
5	Burn	+	+	+	+
6	Sputum	+	+	+	+
7	Wound	+	+	+	+
8	Burn	+	+	+	+
9	Burn	+	+	+	+
10	Sputum	+	+	+	+
11	Wound	+	+	+	+
12	Wound	+	+	+	+
13	Sputum	+	+	+	+
14	Burn	+	-	-	-
15	Sputum	+	+	+	+
16	Sputum	+	+	+	+
17	Wound	+	+	+	+
18	Burn	+	+	+	+
19	Wound	+	+	-	+
20	Burn	+	-	-	-

APPENDIX 5. Distribution of biofilm genes (*algD*, *pslD* and *pelA*) among clinical isolates

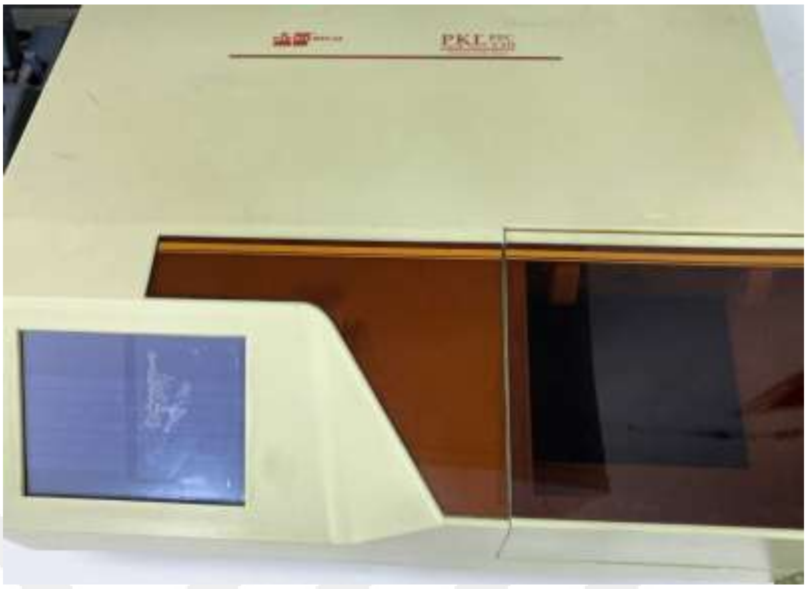
Lab No.	Sample type	Age	Sex	Biofilm intensity	DNA Result	PCR *algD* 593bp	PCR *ps1D* 369bp	PCR *Pe1A* 786bp
1	Wound	30	Male	Weak	+	+	-	+
2	Burn	52	Female	Strong	+	+	+	+
3	Sputum	46	Male	Moderate	+	+	+	+
4	Wound	40	Male	Moderate	+	-	-	-
5	Burn	55	Male	Moderate	+	+	+	+
6	Sputum	48	Female	Strong	+	+	+	+
7	Wound	35	Female	Moderate	+	+	+	+
8	Burn	49	Male	Moderate	+	+	+	+
9	Burn	58	Male	Moderate	+	+	+	+
10	Sputum	49	Male	Moderate	+	+	+	+
11	Wound	51	Male	Moderate	+	+	+	+
12	Wound	45	Male	Strong	+	+	+	+
13	Sputum	46	Female	Strong	+	+	+	+
14	Burn	61	Female	Moderate	+	-	-	-
15	Sputum	40	Male	Weak	+	+	+	+
16	Sputum	71	Female	Weak	+	+	+	+
17	Wound	51	Male	Strong	+	+	+	+
18	Burn	44	Female	Strong	+	+	+	+
19	Wound	48	Female	Strong	+	+	-	+
20	Burn	33	Male	Moderate	+	-	-	-

APPENDIX 6. Microtiter plate with ethanol



APPENDIX 7. Stainless Steel laboratory thermostat incubator DH3600BII

APPENDIX 8. Automatic ELISA reader PKL PPC 230



APPENDIX 9. Multichannel micropipette

APPENDIX 10. VITEK 2 complex system

APPENDIX 11. AST (ANTIBIOTIC SUSCEPTIBILITY TEST CARDS)

APPENDIX 12. ***Pseudomonas aeruginosa*** **sub culture on mackonkey agar media**

APPENDIX 13. Pyocyanin pigment phenotype

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