Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Kerbala
College of Applied Medical Sciences
Department of Clinical Laboratories

Forensic Study of Some Immunological and Bacteriological Parameters Among Burned Victims

A thesis

Submitted to the Council of the

College of Applied Medical Sciences – University of Karbala

in Partial Fulfillment of the Requirements for the Degree of Master in

Clinical Laboratories-Forensic science

Submitted by

Haider Naama Ouda Al-Jubouri

B. Sc. College of Science / University of Babylon (2008)

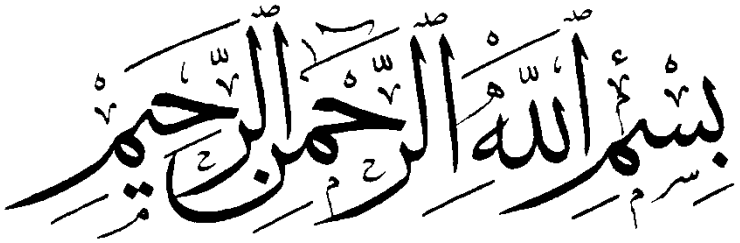
Supervised by

Assis. Prof. Dr.
Israa Saeed Abbas

Prof. Dr.
Ali Hmood M. Al-Saadi

November / 2021 A.D

Dhul Qa'dah / 1443 A.H



{ وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيما }

صدق الله العلي العظيم

سورة النساء / الآية 113

Supervisors Certification

We certify that this thesis entitle (Forensic Study of Some Immunological and Bacteriological Parameter Among Burned Victims) was prepare under supervision at the department of Clinical laboratories, College of applied medical science , University of Kerbala as a partial requirements for the degree of master in Clinical laboratories.

Signature
Assis. Prof.
Israa Saeed Abbas
College of Applied Medical Sciences
University of Kerbala
Date: / / 2021

Signature
Prof.
Ali Hmood M. Al-Saadi
College of Science
University of Babylon
Date / / 2021

In view of the available recommendation, I present this thesis for evaluation by the Examining Committee.

Signature
Prof. Dr. Hadi Rasool Hassan
Head of Clinical laboratories Department College of applied
University of Kerbala
/ / 2021

Dedication

To who I carried his name with pride …
To he who gave me confidence and was the blessing of the father, **may Allah have mercy on him**, **my dear father**.
To my first teacher from whom I drew ethics and morals ..to the warm embrace and the beach of tenderness … that spared her life for me … **my dear mum may Allah have mercy on her.**
To my support in life … **my brothers and my sisters**.

To my wife who sacrificed and still sacrificing for my happiness.
To the candle of my life and the light of my path …..
My daughter **(Sama)**.
To everyone who loves science and teaches it …..
I dedicate the fruit of my humble effort …

Haider 2021

Acknowledgments

In the name of “Allah” who deserves all praise and thanks for inspiring me with the willingness , strength, and patience to establish this work, and peace and pray upon his messenger, The head of the creatures, Prophet Mohammed, his family and his companions.

I would like to express my sincere gratitude and appreciation to my brilliant supervisor Professor. Dr. Ali Hmood M.Al-Saadi and Assis Prof Dr. Israa Saeed Abbas for the scientific guidance, encouragement, support, concern, and kind advice and valuable advices during the work.

I would like to thank The Deanery of College of Applied Medical Sciences / University of Kerbala, for their kind cooperation.

I would like to thank ministry of interior for permission to fulfill my M.Sc study generally, especially the brigadier, Mahmoud Shaker Rahim.

Thanks to University of Babylon, (College of Science-DNA Laboratory) for letting me work for them, represented by Dr. Mona Najah, Dr. Rabab Imran and Dr. Noor Mohamed Salman. very grateful to Dr. Hawraa Natiq Kabroot for her continued assistance.

I extend my thanks to the medical staff in the burn unit and the microbiology unit at Imam Al-Sadiq Hospital, peace be upon him Dr. Alia Muhammad, Dr. Zahraa Qais, Dr. Rana Hadi and the medical staffat the burn center in Al-sadr medical city in Najaf and the medical staff in burn unit at Al-Hussen teaching hospital in kerbala.

Last but not least, thanks to everyone who helped me by every way, even if it was a good word.

Haider 2021

Summary

Pseudomonas aeruginosa is Gram-negative, facultative an aerobic rods, motile by polar flagellum, non-fermentative, non-sporulating opportunistic pathogens that cause a high rate of mortality and morbidity in hospitalized patients with compromised immune systems. It has the ability to infect almost all tissues of the body as a result of its possession of a large variety of virulence factors that contribute significantly to the pathogenicity of the host, so the rapid detection of these bacteria plays a crucial role in controlling the diseases caused by it, especially in burn injuries.

A total of 100 swab samples were collected from hundred burned patients admitted to three different hospitals in three governorates, The burn unit at Imam Al-Sadiq Hospital (peace be upon him) in Babylon, the burn center at Al-Hussein Teaching Hospital in Kerbala and the burn center in Al-Sadr Medical City in Najaf, during the period from December 2020 to April 2021. These swabs samples was used to investigate the spread of *P. aeruginosa* and study some virulence factors. In addition to that, 40 blood sample was collected from some of the burned patients in addition to 30 blood sample was collected from apparently healthy individuals. Blood samples was used to investigate the serum level of IL-8 by ELISA.

Swab samples were cultivated on Blood agar, MacConkey agar, and Pseudomonas chromogenic agar. The diagnosis of the isolates was confirmed along with antibiotic sensitivity testing by using the vitek2 compact system. Molecular diagnosis by PCR technology, study of DNA genetic sequence and hypothetical protein structure for *P. aeruginosa* isolates was done by adopting four genes: *lasI*, *lasR*, *oprI* and *oprL*.

The results showed that the percentage of burned males was 46%, while the percentage of females was 54%. The highest rate of infection was in children age group 1-10 years (42%), followed by the age group 11-20 with 27%, and the

lowest infection rate was in the age group 51-60 and the group above 61 with 1%. There were a high significant difference among age groups ($P \leq 0001$).

The results indicated that there was a significant difference ($P \leq 0.002$) among the diagnosed cases of injuries in regards to the type of burn, as the highest percentage of burn injuries was from liquid burns by 57%, followed by burns by fire at 41%, and the lowest percentage of injuries was for electric shock burns by 2%. In addition, There were significant difference concerning the degrees of burn rates, Second degree burns were 54% and third degree burns were 45%.

The results of the study showed that the most common bacteria from burn was *P. aeruginosa* 67.6%, followed by *Klebsiella* 12.2%, *Eschreichia coli* 10.8%, Proteus 4%, and the least were *Acinetobacter* 2.7%, and *Citrobacter* 2.7%.

The results also showed a high resistance rates of *P. aeruginosa* to most antibiotics, the highest resistance rates was show against Cefazolin (100%) followed by Tigecycline (96%), Imipenem (86%), Amikacin (86%), Levofloxacin (86%), Gentamicin (84%), Cefepime (74%), Ceftazidime (56%), Ciprofloxacin (4%) while all samples showed high sensitivity (100%) to Piperacillin/Tazobactem.

Regarding molecular technique for *P. aeruginosa* isolates, the results showed that all isolates were have *oprI*, *oprL* genes (100%) and 98% of these isolates have *lasI* gene and 88% of isolates have *lasR* gene within the genotype of *P. aeruginosa* isolates under study.

The alignment sequencing was performed to study the degree of similarity with global strains, compared all DNA sequencing results for *P. aeruginosa* isolates with the NCBI database, alignment results showed nucleotide sequence ratios similarity with the ratios of the nitrogenous bases stored in the gene bank to a high percentage. The study of the hypothetical protein structure (insilico) showed

the presence of gaps at the end of the C-terminal and the transformation of β-sheet proteins due to some genetic changes in the genes of *lasI*, *lasR*, *oprI* and *oprL*.

The results of the current study showed that the concentration of IL8 in the group of burn patients had a significant increase in the serum level of second and third degree patients ($P \leq 0.001$) and there was significant increase IL8 level among patients with bacterial infection in comparison to growth negative swabs in the same burn degree at ($P \leq 0.05$).

This study concluded that the prevalence of *P. aeruginosa* is high among clinical samples and it was found that *P. aeruginosa* isolates are resistant to most antibiotics except Piperacillin/Tazobactem which was sensitive to it (100%). The presence of *lasI*, *lasR* and *oprI*, *oprL* genes among *P. aeruginosa* isolates might possibly considered as an alternative methods for diagnosing this bacteria by molecular methods based on PCR and DNA sequencing technology. Moreover, the use of IL-8 as a biomarker for inflammatory processes caused by burns, especially among patients with bacterial infection in the same degree.

Contents

List of Tables

List of Figures

List of Abbreviations

Symbol	Description
16S-rRNA	Sixteen S ribosomal ribonucleic acid
24 hrs	24 hour
AACs	Acetylation, by aminoglycoside acetyltransferases
Ab	Anti-body
AHLs	N-acyl homoserine lactones
AIDS	Acquired immune deficiency syndrome
AIs	Auto inducers
AM	Ampicillin
AMEs	Aminoglycoside-modifying enzymes
AN	Amikacin
ANTs	Adenylation,by aminoglycoside nucleotidyltransferases
APHs	Aminoglycoside phosphortransferases
AST	Antibiotic susceptibility test
BLAST	Basic local alignment search tool
BLI	β-lactamase inhibitors
Bp	Base pair
BWEs	Burn wound exudates
C°	Degrees celsius
CAZ	Ceftazidime
CBC	Complete blood count
CF	Cystic fibrosis
CIP	Ciprofloxacin
CNS	Central nervous system
CRC	Cyclic redundancy check errors
CRI	Chronic respiratory infection
CRO	Ceftriaxone
CXC	Cysteine X cysteine

CXCL8	chemokine which belongs to cysteine X cysteine
CXCR1	chemokine receptor type 1
CXCR2	chemokine receptor type 2
CZ	Cefazolin
D.W	Distil water
dCTP	Deoxycytidine triphosphate
ddH2O	double-distilled water
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleoside triphosphate
eDNA	Extracellular DNA
EDTA	Ethylene diamine tetra acetic Acid
Eliza	Enzyme-linked immunosorbent assay
EP	Eppendorf
ESB	ESBL
ESBLs	extended-spectrum β-lactamases
ESKAPE	*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.
ETP	Ertapenem
Exo S	Exotoxin S
Exo T	Exotoxin T
Exo U	Exotoxin U
Exo Y	Exotoxin Y
F	Degrees fahrenheit
FEP	Cefepime
FOX	Cefoxitin
FT	Nitrofurantion
G-CSF	Granulocyte-colony stimulating factor
GM	Gentamicin
GN	Gram-negative
H2S	Hydrogen sulphide
HAQs	4-hydroxy- 2-alkylquinolines
HIV	Human immunodeficiency
HRP	Horseradish Peroxidase
HSL	Homoserine lactone
IATS	International antigenic typing scheme
ICUs	Intensive care units
ID	identification
IFN	I interferon
IL10	Interleukin-10
IL-8	Interleukin-8
ILs	Interleukins

IMP	Imipenemase
IPM	Imipenem
IV	Intra venous drug
kDa	KeloDalton
lasI	acyl-homoserine lactone synthase
lasR	transcriptional regulator
LEV	Levofloxacin
LPS	Lipopolysaccharides
MBLs	Metallo-beta-lactamases
Mbp	Million base pair
MDR	Multi drug resistance
MIC	Minimum inhibitory concentration
µl	Micro liter
MLST	Multi-locus sequence typing
MOF	multiple organ failure
MW	Molecular weight
NCBI	National center for biotechnology information
O.D	Optical density
OprD	Outer membrane porin D
OprF	Outer membrane porin F
oprI	Outer membrane lipoprotein
oprL	Outer membrane (peptidoglycan associated lipoprotein)
OPRs	outer membrane proteins
P. aeruginosa	*Pseudomonas aeruginosa*
PAGI	*P. aeruginosa* genomic island
PAMP	pathogen-associated molecular patterns
PAPI	*P. aeruginosa* pathogenicity islands
PBPs	penicillin-binding proteins
PCR	Polymerase chain reaction
Pel	Third polysaccharide
PHB	Poly- hydroxyl butyrate
Psl	Polysaccharide
QS	Quorum sensing
QSIs	quorum sensing inhibitors
RhlR	transcriptional regulator
ROS	Reactive oxygen species
rRNA	Ribosomal RNA
SNP	Single nucleotide polymorphism
SPM	Sao Paulo metallo-lactamase
SXT	Trimethoprim/ Sulfamethoxazole
TBE	Tris-Borate-EDTA
TBSA	Total body surface

TE	Tris-EDTA buffer
TGC	Tigecycline
TNF	Tumor necrosis factor
TNFR1	Tumor necrosis factor receptor 1
TNF-α	Tumor necrosis factor alpha
ToxA	Exotoxin A
TSI	Triple sugar iron
TZP	Piperacillin/Tazobactem
UV	Ultraviolet
VAP	Ventilator-associated pneumonia
vatM	Vacuolar ATPase transmembrane subunit
VIM	Verona integron-encoded metallo-lactamase
WGS	Whole genome sequencing

List of Appendices

Chapter One

Introduction and Literatures Review

Introduction and Literatures Review

Introduction

Burn is considered globally to be a one of major public health concern and is in high risk of nosocomial infections denatured and dead, moist tissue makes the burn wound sensitive to *P. aeruginosa* infection, breakdown of skin barriers, reduced immunity, and prolonged hospital stays are significant factors that to make burn wound infected with these opportunistic pathogens (MDR), multidrug resistant *P. aeruginosa*" MDR responsible for 4-60% nosocomial inflammation of different countries as the cause of death and morbidity in burning unit patients (Hasan *et al.*, 2019).

Pseudomonas aeruginosa possess a highly capacity to form biofilms that are cell communities enclosed in an extracellular self produced matrix which protects cells from antibiotics and host immune responses. Biofilm can increase *P.aeruginosa* infection in comparison with planktonic bacterial cells and increase the degree of antibiotic resistance such as (β-lactam and carbapenem antibiotic group) and its capacity to develop more resistance to several groups of antibiotics including aminoglycosides and fluoroquinolones (Schaible *et al.*, 2020).

The QS (Quorum sensing) gene are a type of cell-cell communication, PCR is a very precise and quick method in the detection of *P. aeruginosa* virulence factor genes , these different virulent factors to assist in its pathogenicity and to evade from host defense mechanisms (Fadhel and Hamim, 2020), the (Quorum sensing) QS-essential genes and it's a type of cell to cell communication of the bacterium are the *lasI* and *lasR* genes. In addition the lipoprotein (*oprL*) and outer membrane lipoprotein (*oprI*) associated with peptidoglycan (Secretory Pump), which is associated to the *P. aeruginosa* outer membrane protein. Both genes have the intrinsic resistance to *P.aeruginosa* antibiotics (Hasan *et al.*, 2019).

Burn injury causes significant activation of inflammation and cytokine release but the acute and sub-acute inflammatory response time resolution has not yet been completely delineated. Interluken-8 (IL-8) is a chemical released by macrophages at the beginning of post-injury, an important inflammatory protein where neutrophil and other immune cells are recruited at the site of infection, interlukien-8 also is released by epithelial cells, smooth airway muscle cells, and endothelial cells in addition to macrophages. This chemokine has been shown to participate in many cells, including cell proliferation, remodeling of tissues and angiogenesis (Bergquist *et al.*, 2019).

The IL-8 acts rapidly, suggesting that early follow-up of this chemokine may provide rapid infection information. Based on the role of IL-8 as an inflammatory and multi proliferating mediator, that systemic levels of IL-8 can be used in serious burning patients to predict infections dangerous situations (Kraft *et al.*, 2015).

The IL-8 play a crucial role in burn injury and the serum IL-8 level could be a biomarker for mortality in burn patients. Inflammatory reaction is caused by burn injury in the early hospital phase. Subsequently, leads invasion of microorganisms for burn wounds to exacerbates inflammation, cytokines release. The excessive release of inflammatory cytokines results in serious systemic inflammation that induces tissue damage and vascular endothelial injury and that can progress to multiple organ failure and eventually lead to the patient's death (Matsuura *et al.*, 2019).

Aim of Study

This study aimed to detection the molecular markers of *P. aeruginosa* (*lasI, lasR, oprI, and oprL*), and antibiotic resistance in addition to study association of IL-8 and with correlation of bacterial infection among burned victims. This aim will be achieved through the following **objectives :**

1. Study the association of some socioeconomic factors (e.g age, sex) with the burning occurrence.
2. Isolation and identification of *P. aeruginosa* bacteria from first, second, and third degree of burn victims on Pseudomonas Chromogenic Agar.
3. Study the incidence rates of *P. aeruginosa* infection in burns patient with their susceptibility patterns by using VITEK2 system.
4. Detection of molecular markers (*lasI*, *lasR*, *oprI* and *oprL*) using PCR DNA sequencing technique .
5. Determination of serum IL-8 levels of burned subjects by using ELISA test and there correlation to *P. aeruginosa* infection.

1. Literatures Review

1.1. Burns

A burn is an injury to the organic tissues resulting from a direct or an indirect effect of heat or by flame and hot liquids or contact with hot objects or exposure to corrosive chemicals, radiation and contact with electrical current. Burns lead to the destruction of the skin layer, which is an important tool against the invasion of microbial (Pereima *et al.*, 2001; Siviero Do Vale, 2005). Burn injuries is an important health problem in many countries in the world, as the risk of this injury is influenced by a number of factors, including the extent and depth of the burn, various host factors and virulence factors for bacterial colonies associated with burns (Church *et al*., 2006).

Bacteria are among the most common pathogens of burns, and these bacteria form the biofilms of many types on burns within 48-72 hours of injury, these microorganisms travel from the patient's own skin (hair follicles, sweat glands, gastrointestinal tract, and respiratory system, as well as through contact with health care workers and the external environment). Heat injuries lead to the destruction of the skin layer, which usually prevents the invasion of microorganisms during the first weeks after the injury, With burning, studies indicate that 75% of deaths are caused by burn injuries, which are related to infections, on the other hand, the pattern of injury varies from patient to patient, so the various bacteria associated with burn cases may change dramatically through (Rajput *et al.*, 2008). Burn wounds are a complex traumatic event of several systemic and local effects, affecting many organ systems after the skin. The pathology of the burns victim appears the high the complexity of inflammatory response reactions other hand (Çakir and Yeğen, 2004). Usually, the accidents of criminal burns happen due to failure to prevent them. This trauma has an element of many incidences and added to it in suspicion of a crime (Peranantham *et al*., 2014).

1.2. Type of burns

1.2.1. Thermal burns

They are caused by flashed light, flame, blazing, or contact with a hot surface and include:

1.2.1.1. Hot liquids and fire

The explosions of flammable liquids, natural gas, propane, gasoline results into flash burns (Masood *et al.*, 2016).

1.2.1.2. Flame burns

Flame burns are usually caused by prolonged exposure to intense heat, frequently associated with clothing ignited by stoves and heaters, improper use of flammable liquids, automobile accidents and house fires (Masood *et al.*, 2016).

1.2.1.3. Scald burns

Scalds involve burn hot liquids like water, oil, grease or tar. A deep burn can be caused by water at 140 degrees (F) in 3 seconds, but the same injury will be resulted in just one second at 156 degrees (F) (Masood *et al.*, 2016).

1.2.1.4. Contact burns

They are caused by hot coals, plastics, metals or glass. They may be painful and deep (Masood *et al.*, 2016).

1.2.2. Chemical burns

They are caused by exposure to reactive chemical substances such as strong acids or alkalis (Gnaneswaran *et al.*, 2015).

1.2.3. Electrical burns

Passage of electrical current from an electrical outlet or appliance through the body may result into the electrical burn (Buja *et al*., 2010).

1.2.4. Radiological burns

Alpha, beta or gamma radiations are responsible for radiological burn. To stop the injury process there is a need of decontamination procedure for the people exposed to these types of radiation (Masood *et al.*, 2016).

1.3. Classification of burns degree

1.3.1. First degree burns

The epidermis is involved in first degree burns which are like sun burn, erythematous, sore, and coarse. The minor thermal injury or exposure to severe ultraviolet radiation may cause first degree burns. Their healing time is 5 to 10 days (Lloyd *et al.*, 2012).

1.3.2. Second degree burns

It is further divided into two categories:

1.3.2.1. A superficial partial thickness burns

They usually invade into the superficial papillary dermis. They are characterized with reddish blisters. When pressure is applied, the blisters may shrink and their healing time is 2-3 weeks (Toussaint and Singer, 2014).

1.3.2.2. Deep partial thickness burns

They penetrate the reticular dermis and are yellow or white in color, rough in nature and are very painful. They require more than 3 weeks for complete healing (Toussaint and Singer, 2014).

1.3.3. Third degree burns

They damage both inner and outer layers of the skin, that’s why this is the most severe type of burns. They are white in color and usually non-achy. Just a few such burns are cured of themselves which is a long process (Shank *et al.*, 2009).

1.3.4. Fourth degree burns

They invade into the harmed muscles, ligaments, tendons, nerves, blood vessels, and bones, through the skin. For this type of burns, severe medical emergency care is required. They're black and scorched (Vadukul, 2012).

1.4. *Pseudomonas aeruginosa*

The genus of *Pseudomonas* is gram-negative, aerobic, rod-formed and has unipolar pinion (Fariñas and Martínez-Martínez, 2013). *Pseudomonas* capable of producing pigments a positive oxidase reaction (Gellatly and Hancock, 2013). As well as *P. aeruginosa* is a high intrinsic antibiotic resistance, together with its rapid ability to gain new antimicrobial resistance this pathogen is an increasing problem for the pathology of infectious diseases, especially if the nosocomial originates, no medical trials exist to investigate the potential survival factors of hospitalized patients with *P. aeruginosa* urinary tract infections, the mortality of these patients except bacteremia is not understood (Horino *et al.*, 2012). In critically diseased and weakened patients, particularly in ventilation related pneumonia (VAP) and bloodstream infections, urinary tract, intra-abdominal wounds, skin soft tissue (Lynch *etal*., 2017). *P. aeruginosa* is one of six ESKAPE pathogens that is the main cause of infectious nosocomial and is a global menace, as it becomes increasingly immune to all antibiotics available (Tümmler, 2019) .

The *Pseudomonas* genus consists of over 120 species, which are pathogenic to animals and humans and are widespread in moist environment such as water and soil ecosystems. *P. aeruginosa* is most often associated with human infections in the genus of *Pseudomonas*. The bacterium is considered an opportunistic pathogen, causing mainly nosocomial infections in patients affected by an immune problem. Existing knowledge of *P. aeruginosa* pathogenesis is obtained mainly by studying clinical isolates, particularly those that cause chronic pulmonary infection in patients with cyst fibrosis. Nosocomial infections

most often linked to *P. aeruginosa* include ventilator-related pneumonia, catheter-related urinary-tract infections, serious burn patient wound infections, and multifactorial septicemia with pathogenesis. The bacterium is also able, via the type III secretions system, to produce many toxins. as well as Secretion of enzymes, proteins and elastases, phospholipase C and siderophores (Streeter and Katouli, 2016). The pathogenesis of these bacteria is challenging and is distinguished by the capacity for virulence and biofilm growth to lead to nosocomial infection (Silva *et al.*, 2019).

Pseudomonas aeruginosa demonstrates tolerance to a broad range of antimicrobials and expresses a variety of molecular epidemiology in different groups of antibiotic agents, such as β lactams, fluoroquinolones, tetracycline and aminoglycosides. Although the external membrane is poor in permeability, its hydrophilicity and unspecified behavior to small molecular transport. The mechanism for the resistance of *P. aeruginosa* to different chemical agents is due to the complex genes encoded chromosomally, different strains with inherent biofilm ability of *P. aeruginosa* further improve the resistance under different environmental factor (Mohanty *et al*., 2021).

Moreover, the bacteria of *Pseudomonas* in the environment are normal. It is present in many ways artificially and environmentally friendly. You can get to it in soil, fresh water, sea and in many parts of the human world. *Pseudomonas* goes from an enormous variety of bacteria to a smaller population of bacteria. This led to the transition of many bacteria to other genera, families and environmental types. In the past 100 years *Pseudomonas* has undergone many taxonomic changes with some characteristics that have become smarter and more orderly (Özen and Ussery, 2012). This bacterium adapts greatly to its surroundings, which also choose to support the persistence of bacteria, The development of surface or cell adhesive bacterial biofilms associated with enhanced immune and antibiotic clearance are of a clinically important temporal

adjustment, Extensive research has shown that bacterial flagella motility facilitates biofilm formation which is subsequently nonmobile in bacteria , However recent evidence has shown that nonattached antibiotic resistant bacterial aggregates can develop and are documented in the context of lung infections, otitis media, non-healing wounds and soft tissue fillers, which do not comply with surface attachment (Demirdjian *et al.*, 2019).

1.5. Taxonomy of *Pseudomonas*

Pseudomonas genus was described firstly in 1894, since that time, many species were isolated from this genus when the first trials for classification of *Pseudomonas* were made according to diagnostics characteristics (Peix *et al*., 2009). Gilardi put the first system to classify microorganisms related to the family of *Pseudomonadaceae* depended on phenotypic characteristics and divided it into the main seven groups: *Pseudomallei, Alcaligenes, Stutzeri, Fluorescent, Diminuta, Facilis-delafieldii and acidovorans* (Li *et al*., 2010). Study of Mac Aogáin *et al*., (2012), mention that the best-characterized groups to classification of Pseudomonas genus are subdivided according to properties such as the presence of poly-hydroxyl butyrate (PHB), the production of fluorescent pigment pathogenicity; the presence of arginine dihydrolase, glucose utilization and production of Di-aminoacetophenone as a group like odors (Von Bodman *et al*., 2008).

Recently, a lot of well-educated techniques using for molecular analysis come to be available; five groups were extensively refined from this genus. It was identified according to five individual rRNA groups within the genus. Those rRNA genes are highly preserved genes, and the 16S-rRNA sequences serve as the main genetic marker molecules in bacterial phylogeny with the extra information providing by the 23S-rRNA genes in addition to the sequences of genes coding for highly conserved proteins. *Pseudomonads*, based on their 16S-rRNA sequences, are classified as members of the group of γ- Proteobacteria

(Kung *et al*., 2010). Table (1-1) shows the classification of *Pseudomonas aeruginosa*.

Table (1-1). The Classification of *Pseudomonas aeruginosa* (Kim *et al*., 2012).

Domain	Bacteria
Phylum	Proteobacteria
Class	Gamma proteobacteria
Order	Pseudomonadales
Family	Pseudomonadaceae
Genus	Pseudomonas
Species	*Pseudomonas aeruginosa*

1.6. Characteristics of *P. aeruginosa*

Pseudomonas aeruginosa belongs to genus Pseudomonas. It easily grows on regular media. This species creates several bacterial pigments such as pyocyanin More than half of the clinical isolates contain pyocyanine from the blue-green pigment (El Solh and Alhajhusain, 2009). These bacteria which are found in natural surroundings, might be initiated in a planktonic form or in a biofilm, attached to some surface or substrate, as a unicellular organism, actively swimming using its flagellum (Wei and Ma, 2013).

Pseudomonas aeruginosa has various types of motility, which enable it to travel on fluid and solid surfaces (swimming, twitching and swarming). *P.aeruginosa* produces various factors of virulence that help to colonize a host. These include protease enzymes, pili, exotoxin A, pigments, lipase, haemolysin, histamine, exoenzyme S, leukocidin and rhamnolipids. These factors of virulence help *P. aeruginosa* adhere and invade their host by damaging the host's immune reactions and creating antibiotic barriers (Schaber *et al.*, 2004).

Pseudomonas aeruginosa has a width of 0.5 to 1 mm and a length of 1.5 to 5mm. Almost all strains have a single polar flagellum that allows them to move. Positive oxidase checks, the ability to develop under anaerobic conditions through the presence of an alternate terminal electron acceptor such as nitrite or

arginine, and the ability to grow at 37-42°C are all phenotypic characteristics of *P. aeruginosa* isolates (Hashem *et al.*, 2018).

Pseudomonas aeruginosa is a generically isolated bacterium of various aqueous solutions, like designers, soap, eye drops, from hospitalized patients. It can also found in respiratory devices, bathhouses, and produces the pigment pyocyanine, which is not fluoresce (Todar K., 2004; Bekele *et al.*, 2015). As well isolate from various environments such as soil, plants, and different aquatic environments. Human wastewater is one of the most common sources for the isolation of *P. aeruginosa*. It can grow in distilled water", which is substantiation of its minimal nourishing needs. In the laboratory, the pretentious medium for growth of *P. aeruginosa* contains ammonium sulfate as a source of nitrogen and acetate as a source of carbon (Hoiby, 2011).

Pseudomonas aeruginosa stands a range of physical conditions, including temperature, even though the optimum temperature for its growth is 37°C. It is accomplished by growing at temperatures as high as 42°C (Ubonchonlakate *et al*., 2012).Furthermore *P. aeruginosa* can endure the most challenging environments due to its low nutritional requirements and ability to exploit a range of natural and artificial compounds as a carbon energy source. Consequently, it is no surprise that this ubiquitous bacterium can thrive in disinfectants and catheters (Williams *et al*., 2010).

1.7. Nosocomial infection

Hospital infection is one of the most important causes of deaths in burn units, as studies have indicated that a large proportion of deaths are related to this infection due to resistance of pathogens associated with burns to antibiotics (Tayh, 2013). The causes of hospital infection are of internal origin endogenous, which are caused by microorganisms that are part of the patient's natural flora, and of external origin exogenous that gain from the patient's exposure to the hospital environment (Samuel *et al.*, 2010).

The role of contaminated medical devices in the transmission of the healthcare-associated pathogen and environmental surfaces has been well reported (Otter *et al.*, 2015). Previous studies recommend that microbial contamination of those devices and surfaces play the main role in the range of pathogens (Gebel *et al.*, 2013). The ability of microorganisms to remain viable on dry surfaces effective on pathogen transmission, their resistance to disinfectants and the frequency that devices are in contact with patients and healthcare workers or contaminated surfaces (Weber *et al.*, 2010).

The bacteria is one of the pollutants hospitals known, and its existence becomes one of the biggest problems experienced by personals who work in the hospitals such as doctors, nurses, workers (Krogulski, 2008). They are at risk of injury from clinical specimens or wounds exudate, which may be the source of contamination with the bacteria that moves to a group of not infected patients of others injured either by direct contact between patients and staff (Biccard and Rodseth, 2011). Another report showed that bacteria could transform from a variety wet sources in hospitals such as water cycles, faucets, wipers territory, clothing collection containers and soap savers (Vincent *et al.*, 2000).

Pseudomonas aeruginosa is one of the famous bacteria which could be found and caused pollution in the hospitals are isolated from plastic containers that used to carry bandages which were a way for the transfer of bacteria from one patient to another replaced with metal containers possibility sterilized after use (Schechner *et al.*, 2009). These bacteria have the ability to live in some sterile solutions and polluted it, such as cleaning solvents to eyes and physiological fluids and sterile chloride solutions. The difference of contamination percentage between hospital depends on the type of patient treated and methods of supervision, control, and efficiency of medical staff (Irazoqui *et al.*, 2010).

1.8. *P. aeruginosa* Associated wound and burn infection

Pseudomonas aeruginosa is one of the most common pathogens isolated from burn patients throughout the world (Sousa *et al.*, 2018). *P. aeruginosa* is an opportunistic bacterium associated with healthcare infections in intensive care units (ICUs), ventilator-associated pneumonia (VAP), surgical site infections, and burns (López-Jácome *et al.*, 2019). Burn wounds infection is a great problem because it may lead to death in 75% of patients with injuries (Santucci *et al.*, 2003). The undamaged human skin surface is vital to protect the homeostasis of bodily fluid, thermo-regulation and host infection control. As the first line of defense, the skin is equipped with arrange of immune mediators capable of engaging inflammatory cells to support neutralization and clearance of microbes, it is one of the most important pathogens involved in burn infections (Steinstraesser *et al.*, 2004; Rafla and Tredget, 2011). *Pseudomonas aeruginosa* is a common nosocomial pathogen in burn patients, and acquires antibiotic resistance rapidly; thus the most successful method to fight infection is the efficient therapeutic approach (Ranjbar *et al.*, 2019). The high prevalence and gradual increase of MDR, particularly in burn centers *P. aeruginosa* seriously threats the patients with severe burn injure (Dou *et al.*, 2017; de Almeida Silva *et al.*, 2017).

Burn wound infections are one of the most important complications that occur after burn injuries and may be associated with serious clinical complications and increased morbidity and mortality (Turner *et al.*, 2014). Burn injury compromises the primary barrier of the host, the skin, which immediately places the host at risk for infection (Lopez *et al.*, 2017). Burn wounds are major public health problems all over the world. Infection is one of the most complicated issues in burn patients, because the skin, a barrier against microbes, has been destroyed and the immunity agents cannot reach the sites of infection.

There is a correlation between the severity of infection and the extent of the burn (Anvarinejad *et al.*, 2014).

This bacterium causes 75% mortality in burned patients as it can establish a persistent infection biofilm, express multiple virulence and antibiotic resistance mechanisms. Some of these virulence factors are proteases such as elastase and alkaline protease, or toxic metabolites such as pyocyanin which is one of the few microorganisms able to produce cyanide, which inhibits the cytochrome oxidase of host cells (López-Jácome *et al.*, 2019). Multiple antibiotic resistant *P. aeruginosa* is a major cause of burn wound infections and inflammation of skin and soft tissue. Because of its resistance to commonly used antibiotics and antiseptics, there is a shortage of therapeutic options for effective treatment *P. aeruginosa* normally affects patients of infections with burn and wound where the primary condition can be more complicated and may also cause bacteremia (Inacio *et al.*, 2014).

1.9. Treatment of *P. aeruginosa*

Among infection caused by Gram-negative bacteria, is *P. aeruginosa* which has a leading role (El Zowalaty *et al.*, 2015). The most common pseudomonal infections in the ICU are ventilator-associated pneumonia, urinary tract infections, blood stream infections, and skin and soft-tissue infections. The susceptibility test is essential guide for the choice of a proper antimicrobial agent. Antimicrobial agents that are generally deemed effective against Pseudomonal infections can be categorised into the following:

1. Penicillin with β-lactamase inhibitors (BLI) (ticarcillin and piperacillin in combination with clavulanic acid or tazobactam).
2. Carbapenems (imipenem, meropenem).
3. cephalosporins (ceftazidime, cefepime).
4. Fosfomycin.
5. Polymyxins (colistin, polymyxin B).

6. Monobactams (aztreonam).
7. Aminoglycosides (gentamicin, tobramycin, amikacin, netilmicin).
8. Fluoroquinolones (ciprofloxacin, levofloxacin).

1.10. Pathogenicity of *P. aeruginosa*

Most of Pseudomonas infections are both toxinogenic and invasive. The critical Pseudomonas infection may be seen as composed of three distinct stages bacterial attachment and colonization, local invasion and disseminated systemic disease. Conversely, the disease development may stop at any stage (Miyata *et al.*, 2003). Pathogenicity of *P. aeruginosa* depends on many virulence factors, including cell-related factors and secreted ones (Karatuna and Yagci, 2010).

Pseudomonas aeruginosa rarely infects healthy tissues, but when defenses are compromised, it can infect virtually all tissues (Morrison and Wenzel, 2015). *P. aeruginosa* is well suited for respiratory tract conditions, particularly in chronic obstructive bronchopulmonary diseases, patients who are hospitalized or immuno-intensive (Driscoll *et al*., 2007). It is a major cause of chronic respiratory infection (CRI). CRI by *P. aeruginosa* is the leading cause of morbidity and mortality in cystic fibrosis (CF) patients and a frequent complication of other respiratory diseases such as chronic obstructive pulmonary disease (COPD) or bronchiectasis (Mahar *et al.*, 2010). According to Chastre and Fagon (2002), *P. aeruginosa* is the main cause in ventilated patients with nosocomial pneumonia.. In neutropenic cancer patients undergoing chemotherapy is a common complication of Bacteraemia with *P. aeruginosa* (Krcmery *et al.*, 2006). Bacteraemia and Septicemia can also occur in patients with immune deficiency-related to AIDS, diabetes mellitus or severe burns (Marra *et al.*, 2006).

Most of these contaminations are innate in hospitals and nursing homes. *P. aeruginosa* is also the third leading cause of hospital-acquired urinary tract infections. These infections can occur via descending or ascending routes and are

usually secondary to urinary tract catheterization, surgery or instrumentation. ‘Swimmer’s ear' (a form of external otitis) caused by *P. aeruginosa* is the predominant and malignant otitis in diabetic patients. Devastating ophthalmic infections, meningitis and brain abscesses can be caused by *P. aeruginosa*. Skin and bone infections can also occur by *P. aeruginosa* after puncture wounds, but it rarely causes infections of the digestive tract. Although, perirectal infections, typical gastroenteritis, and necrotizing enter colitis (Lavery *et al*., 1994).

Pseudomonas aeruginosa causing acute urinary tract infections, respiratory infections, bacteremia, otitis media, folliculitis, keratitis in contact lens wearers, soft tissue infections, and a variety of systemic infections (Hattemer *et al.*, 2013). *P. aeruginosa* is detected in patients with defects in host defenses, such as chronic neutropenia and defects of neutrophil function, hematologic cancers, human immunodeficiency (HIV) acquired immunodeficiency syndrome (AIDS). The infections often are associated with significant morbidity and mortality according to the *P. aeruginosa* ability to adapt to the environmental changes and resistance to antibiotics (Fujitani *et al*., 2017).

1.10.1. Virulence Factors

The Pathogenicity of *P. aeruginosa* is mainly caused by multifactorial virulence factors and genetic flexibility. The *P. aeruginosa* virulence factors are classified into two groups: factors that aid in the colonization of the microorganism to host cells (fimbriae and flagella) and factors that assist in the inhibition of the immune response and invasion of tissue. (Driscoll *et al*., 2007). Moreover, virulence factors substantially depending on bacterial physiology as well as on the strain involved. This organism produces a broad array of toxins and other virulence factors that cause immune evasion, tissue damage, and haemorrhage. The virulence factors can be proteinaceous or chemical, and either cell-associated or secreted. Proteinaceous virulence factors are often secreted through one of the five protein secretion systems in *P. aeruginosa*: type I, II,

III, V and the recently discovered type VI (Mikkelsen *et al.*, 2009). These cell-associated, extracellular secreted virulence factors and type III secretion system were summarized in table (1-2, 1-3 and 1-4), respectively.

Table (1-2). Virulence factors in *P. aeruginosa* and their functions (Brooks *et al*., 2007).

Cell-Associated Virulence Factors	
Virulence factor	**Functions**
Extracellular Slime layer Substance	capsular polysaccharide and associated with the outer membrane complex
Flagella	Motility, attachment of bacteria to host cells
Pili	Motility, epithelial interaction.
Lipopolysaccharide	Epithelial and TLR4 interaction.
Capsule(Alginate)	Epithelial interaction, bacterial protection.

Table (1-3). Extracellular Secreted Virulence Factors in *P. aeruginosa* and their functions (Brooks *et al*., 2007).

Extracellular Secreted Virulence Factors	
Virulence factor	**Functions**
Pyocyanin	host-response , neutrophil apoptosis.
Pyoverdine	Iron chelation, regulation of exotoxin A.
Alkaline protease	Fibrin lysing protease, neutrophil function.
Protease IV	Degradation of host tissue and plasma proteins.
Elastase	Degrades tissue and plasma proteins, neutrophil function.
Phospholipase C	Surfactant inactivation, neutrophil function.
Exotoxin A	Inhibits elongation factor 2 (protein synthesis).
Neuraminidase	the enzyme acts to release sialic acid(N-acetyl neuraminic acid) from GM1-ganglioside receptors facilitates attachment of pili and increase adhesive with epithelial cells
Dnase	acts on DNA of host cells and inhibition of genetic machinery of phagocytic cells
Urease	Responsible for the production of renal stone. In gastrointestinal tract infections urease protect *P.aeruginosa* against stomach pH

Table (1-4). Type III Secretion System in *P. aeruginosa* and their functions (Brooks *et al.*, 2007)

Type III Secretion System	
Virulence factor	**Functions**
Exo S	Disrupts cytoskeleton interacts with TLR2
Exo T	Disrupts cytoskeleton.
Exo Y	Adenylate cyclase injected into host cytosol.
Exo U	Major cytotoxin, phospholipase activity.
Quorum sensing:	
las	*las*→AHL → transcriptional activation of virulence genes.
rhl	*rhl*→ HL → transcriptional activation of virulence genes.

1.10.1.1.Biofilm Formation

Biofilms are multi-microcolony microbial communities encased in a self-generated extracellular polysaccharide matrix. The biofilm matrix in *P. aeruginosa* is composed of three distinct exopolysaccharides: alginate, Psl and Pel. Alginate is a polymer consisting of β-D-mannuronic acid and α-L-guluronic acid and has a substantial role in structural stability and protection of biofilm. Psl is a polysaccharide composed of a repeating pentasaccharide, consisting of D-mannose, D-glucose and L-rhamnosePsl aids in the creation of biofilms and the preservation of biofilm structure. Pel is the third form of polysaccharide found in *P. aeruginosa* biofilm and is glucose rich (Wei and Ma, 2013). Biofilm cells are more resistant to antimicrobial agents and other environmental stresses than planktonic cells. (Ghazalibina *et al.*, 2019).

Furthermore, the extracellular matrix, which is made up of extracellular DNA (eDNA), exopolysaccharides, lipid vesicles, and matrix proteins, surrounds the resident bacteria and is a central feature of biofilm communities. Biofilms are common in nature and are an effective survival mechanism for microorganisms in harsh environments. When shaped in industrial settings or on medical devices, they may be beneficial or harmful. As such, research into the formation and

elimination of biofilms is important for any discipline (Azeredo *et al.*, 2017). *Pseudomonas aeruginosa's* ability to form biofilms, which are colonies of cells encased in a self-produced extracellular matrix, shields the bacteria from antibiotics and the immune response of the host (Cassin and Tseng, 2019). As well as *P. aeruginosa's* ability to form biofilms further protects it from antibiotics and the immune system of the host. *P. aeruginosa* is immune to a wide range of antibiotics by itself and can develop resistance to even more, making treatment difficult. *P. aeruginosa* causes a powerful inflammatory reaction during infection (Alhazmi, 2015) .

Pseudomonas aeruginosa is an opportunistic, nosocomial bacterial pathogen that causes the development of defensive communities called biofilms to create persistent infections. Biofilm is a key factor in *P. aeruginosa* virulence and plays a major role in antibiotic resistance and chronic burn wound infections (Banar *et al.*, 2016) . *P. aeruginosa* biofilms contribute to its survival on biotic and abiotic surfaces and pose a significant clinical risk because of its high antibiotic tolerance (Maura and Rahme, 2017). When a biofilm is formed, the bacteria in it are recalcitrant and immune protection against antimicrobials (Pestrak *et al.*, 2019).

1.11. Antibiotic Resistance *P. aeruginosa*

The nature of this organism's inherent resistance to several antibiotics (β-lactam, penem group antibiotics) and its ability to develop further mechanism of resistance to various classes of antibiotics, including beta lactam, amino-glucoses and fluoro-quinolones, makes it difficult to treat infection caused by it. Microbes have implemented various mechanisms to preserve genome plasticity in their molecular evolution. Microbes are mainly used to shape biofilms, quorum sensing, horize gene and enzyme promiscuity for their survival (Pachori *et al*, 2019; Mohanty *et al*., 2021).

As well as excessive use of antibiotics during treatment accelerates development of multidrug-resistant *P. aeruginosa* strains, leads to the inefficacy of empirical antibiotic treatment for this microorganism (Hirsch and Tam, 2010). Generally, the major mechanisms of *P. aeruginosa* used to counter antibiotic attack can be classified into intrinsic, acquired and adaptive resistance figure (1-1).

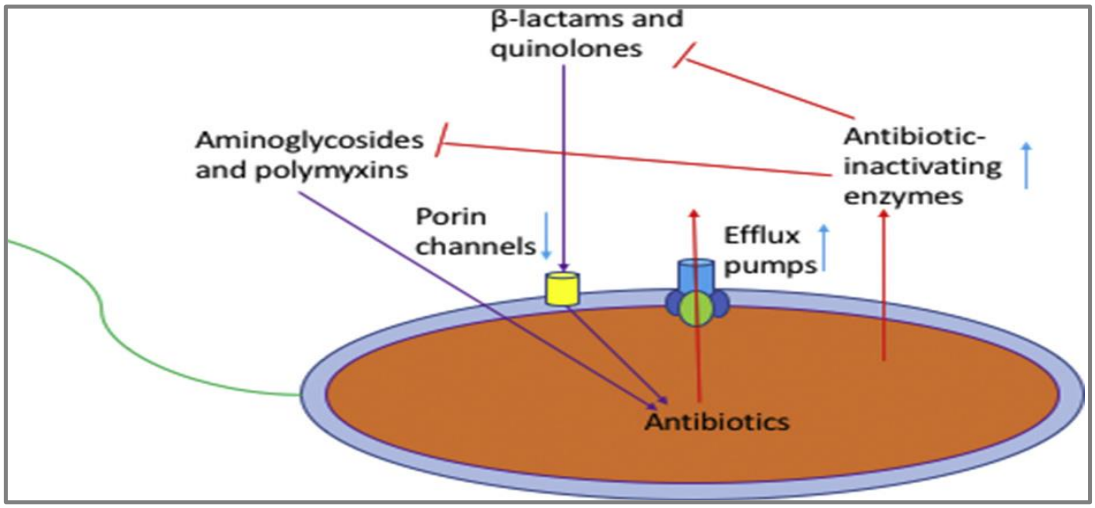


Figure (1-1). A schematic representation of the mechanisms of intrinsic antibiotic resistance in *P. aeruginosa* (Pang *et al.*, 2019).

1.11.1. Intrinsic Resistance

The bacterial species' intrinsic antibiotic resistance refers to their innate capacity to reduce the effectiveness of a particular antibiotic by its own structural or functional properties (Pang *et al.*, 2019). *Pseudomonas aeruginosa* has been shown to have a high degree of intrinsic antibiotic resistance due to restricted outer membrane permeability, efflux systems that pump antibiotics out of the cell, and alginate formation, as well as the transfer of resistance genes and the production of antibiotic-inactivating enzymes such as β–lactamases (Balasubramanian *et al.*, 2013).

1.11.1.1. Outer membrane permeability

In order to meet intracellular goals, most antibiotics used in the treatment of infections of *P. aeruginosa* need to be able to enter the cell membrane (Lambert,

2002). For example, bacteria protein-binding synthesis with ribosomal 30S units is inhibited by the aminoglycoides family of antibiotics like tobramycin, gentamicin and amizacin (Mingeot-Leclercq *et al*., 1999). Quinolone antibiotics such as Ciprofloxacin and Levofloxacin interfere with DNA replication by inhibiting DNA gyrase and topoisomerase IV (Aldred *et al*., 2014). The β-lactam ring is present in the molecular structures of β-lactam antibiotics such as penicillin, cephalosporin, carbapenem, and monobactam. This class of antibiotics blocks bacterial cell wall biosynthesis by targeting the penicillin binding proteins that are enzymes involved in peptidoglycan synthesis (Poole, 2004). Polymyxins are a class of polypeptide antibiotics that attach to the lipopolysaccharides (LPS) on Gram negative bacteria's outer membrane, causing increased permeability and antibiotic absorption. Polymyxin B and polymyxin E, also known as colstin, are the two polymyxins used in clinical practice, and they kill bacteria by induction of a hydroxyl radical-mediated cell death pathway (Zavascki *et al.*, 2007). To enter the bacterial cell, β-lactams and quinolones penetrate cell membranes through porin channels, whereas aminoglycosides and polymyxin promote their own uptake by interacting with bacterial LPS on the outer membrane of Gram-negative bacteria (Lambert, 2002).

1.11.1.2. Antibiotic-inactivating enzymes

Bacterial cells produce enzymes that target antibiotics and render them inactive by chemical modifications such as the addition of specific chemical moieties or complete destruction of the antibiotic molecule. Many antibiotics have chemical bonds such as amides and esters that are susceptible to hydrolysis (Wright, 2005; Munita and Arias, 2016; Arzanlou *et al*., 2017). By enzymes commonly produced by *P. aeruginosa* such as β-lactamases and aminoglycoside-modifying enzymes (Poole, 2005; J Wolter and D Lister, 2013). *P. aeruginosa* has an ampC gene that encodes the hydrolytic enzyme β-lactamase, much like other Gram negative bacteria. This enzyme will sever the amide bond of the

lactam ring, rendering β-lactam antibiotics inactive (Wright, 2005). Furthermore, β-lactamases can be classified into four groups based on their amino acid sequences: A, B, C, and D. Via an active site serine, enzyme groups A, C, and D hydrolysis β-lactams.ClassB β-lactamases,on the other hand,are metallo enzymes that require divalent zinc ions for β-lactam hydrolysis (Bush and Jacoby, 2010).

The class C β-lactamase produced by *P. aeruginosa* has been shown to inhibit anti pseudomonal cephalosporin's, a class of β-lactams (Berrazeg *et al.*, 2015). Extended-spectrum-β-lactamases (ESBLs) have been discovered in some *P. aeruginosa* isolates, which confer a high level of resistance to the majority of β-lactam antibiotics, including penicillins,cephalosporins,and aztreonam (Paterson and Bonomo, 2005; Rawat and Nair, 2010).

1.11.2. Adaptive Resistance

Pseudomonas aeruginosa's adaptive resistance includes biofilm formation in the lungs of infected patients, where the biofilm acts as a diffusion barrier to restrict antibiotic access to bacteria cells, In addition, multidrug-tolerant persister cells that are able to survive antibiotic attack can form in the biofilm; these cells are responsible for prolonged and recurrent infections in cystic fibrosis (CF) patients (Drenkard, 2003; Mulcahy *et al.*, 2010).

1.11.3. Acquired Resistance

Bacteria can gain antibiotic resistance through mutational changes or acquisition of resistance genes via horizontal gene transfer (Munita and Arias, 2016). Moreover, in biofilms may form multidrug resistant cells capable of surviving antibiotic attacks, responsible for prolonged and recurring infections in CF patients (Hainrichson *et al.*, 2007).

1.11.3.1.Resistance by mutations

Mutational changes are ready to cause reduced antibiotic uptake, modifications of antibiotic targets, and overexpression of efflux pumps and

antibiotic-inactivating enzymes; all of which permit bacteria to survive within the presence of antimicrobial molecules (Munita and Arias, 2016). Porins form small water-filled channels within membranes that mediate the diffusion of hydrophilic antibiotics, up to a particular size exclusion limit (Welte *et al.*, 1995). Spontaneous mutations can affect the expression or function of a selected porin, thereby reducing bacterial membrane permeability and increasing antibiotic resistance (Fernández and Hancock, 2012). As mentioned earlier, to stop the intracellular accumulation of toxic compounds, bacteria employ energy-dependent efflux systems to pump the toxic molecules out of the cells (Sun *et al.*, 2014). As a result, clinical isolates of *P. aeruginosa* with overexpressed efflux pumps are less susceptible to antibiotics (Llanes *et al.*, 2004; Cabot *et al.*, 2011; Poonsuk *et al*., 2014 ; Cabot *et al*., 2016).

1.11.3.2. Acquisition of resistance genes

Antibiotic resistance genes can be carried on plasmids, transposons, integrons and prophages, and bacteria can acquire these genes via horizontal gene transfer from the same or different bacterial species (Breidenstein., 2011). Integrons are genetic elements that insert mobile gene cassettes into a specific genetic site via site-specific recombination (Hall and Collis, 1995). and they have been shown to play a critical role in dissemination of antibiotic resistance among *P. aeruginosa* strains (Chen *et al.*, 2009; Odumosu *et al*., 2013; Khosravi *et al*., 2017). The main mechanisms of horizontal gene transfer involve transformation, transduction and conjugation (Arber, 2014). For example, six types of *P. aeruginosa* metallo-β-lactamases (MBLs) have been identified, including imipenemase (IMP), Verona integron-encoded metallo β-lactamase (VIM), and Sao Paulo metallo β-lactamase (SPM), which belong to the class B -lactamases that hydrolyze most β-lactam-based antibiotics (Hong *et al.*, 2015). The genes for these *P. aeruginosa* MBLs have been detected being carried by genetic elements,

including integrons and plasmids (Castanheira *et al.*, 2004; Bonomo and Szabo, 2006; Khajuria *et al.*, 2013 ; Cavalcanti *et al.*, 2015).

1.12. Diagnosis

1.12.1. Phenotypic

Pseudomonas aeruginosa is grown on a MacConkey agar plate to produce colorless colonies (as it does not ferment lactose). Cetrimide agar and pseudomonas Chromogenic agar was selective media for this bacteria. It also has a characteristic fruity smell. *P. aeruginosa* is catalase positive, oxidase positive, and nitrase positive. When grown on TSI medium, it has a K/K profile, meaning that the medium will not change color and sugars fermentation does not occur (Hawkin *et al*., 2010). Moreover, *P.aeruginosa* can de-hydrolyze arginine, unable to decarboxylate lysine or ornithine, unable to produce indole and H2S,Voges-Proskauer and Methyl-Red reactions are negative. It can liquefy gelatin and utilize citrate (Hemraj *et al*., 2013).

1.12.2. Serotyping

Serotyping is the most accepted serotyping method for *P. aeruginosa* which is the International Antigenic Typing Scheme (IATS), and Serotyping frequently used (Lam *et al*., 2011). *P. aeruginosa* identifies 20 different serotypes based on the expression of the O-antigen moiety of the lipopolysaccharide (LPS). However, there are variations in the distribution of *P. aeruginosa* serotypes, with some serotypes being more prevalent in clinical samples. Worldwide, serotypes O:1, O:6, and O:11 have been found more frequently in many clinical settings and among these, serotype O:11 is more often associated with outbreaks in hospitals (Dutra *et al.*, 2014).

1.12.3. Molecular typing

Molecular typing techniques are highly discriminatory, developed over the past decade, and useful for typing strains from patients with a chronic infection

where the bacterial phenotype is unstable. It applies in particular in cystic fibrosis, where patients regularly are infected with the similar strain for more than a few decades, but the bacteria undergo phenotypic alteration. Molecular typing techniques which have confirmed useful in typing *P.aeruginosa*, including electrophoresis, random amplified polymorphic DNA analysis, restriction fragment length polymorphic DNA analysis, multilocus sequence typing and repetitive extra palindromic PCR analysis. These methods are only available in particular laboratories, on the other hand, they should be used when data from the phenotypic typing analysis are ambiguous or when phenotypic methods are unreliable (McAlester *et al*., 2008).

1.13. Genetic content of *P. aeruginosa*

Pseudomonas aeruginosa has one chromosome, and it is genetic material which is rich in guanine and cytosine, in addition to the presence of extrachromosomal genetic element called plasmid (Dettman *et al.,* 2013). The complete genome sequencing of a wild-type *P. aeruginosa* (PA01) strain, achieved in 2000, has to provide a great covenant of useful information, concerning not only its pathogenicity but also its potential for antibacterial resistance, with 5570 open reading frames. *P.aeruginosa* genome is among the major genomes in the prokaryotic world, which encodes an unusually high proportion of proteins involved in regulation, virulence functions, and transport, which may explain the high versatility and adaptive capacity of this organism. Furthermore, 0.3% of the total genes code for proteins implicated in antimicrobial resistance. Also, the genome is highly flexible, with 10% of genes organized in ‘pathogenicity islands’ (PAGI) including flexible genes coding for virulence factors. Moreover, with the ability to easily acquire large mobile genetic elements (integrons) encoding for antimicrobial resistance genes (Brugha and Davies, 2011). The big size and the complexity of this genome are probably the bases for the capacity of *P. aeruginosa* to thrive in diverse environments. The

infect a variety of body sites, and resist (after the acquisition of the necessary genes or intrinsically) a large number of antimicrobial agents (Chatterjee *et al.*, 2016). The genome size of *P. aeruginosa* differs from 5.2 to 7.1 Mbp**.** This degree of difference has important implications for the methods used to study the evolution and epidemiology of this organism.

1.13.1. *OprL* and *OprI* Genes

There are several extracellular and cell associated virulence factors that may lead to its Pathogenicity. The colonization of these factors can cause blood stream invasion, extensive tissue damage and dissemination. Some genes, normally encode and participate in the virulence factors are, toxA, *exoS, exoY, exoU, oprL, oprI, lasA, lasB, oprD, plcH, plcN* and *nan1* etc (Haghi *et al.*, 2018).

Pseudomonas aeruginosa possesses a variety of virulence factors that may contribute to its Pathogenicity such as outer membrane proteins (*oprI* and *oprL*) which play important roles in the interaction of the bacterium with the environment as well as the inherent resistance of *P. aeruginosa* to antibiotics where the consequence of the presence of these specific outer membrane proteins that have been implicated in efflux transport systems that affect cell permeability (Nikaido, 1994). As these proteins are found only in this bacteria, they could be a reliable factor for the rapid detection of *P. aeruginosa* in clinical samples (De Vos *et al.*, 1997).

Pseudomonas aeruginosa having great diversity and capable of causing life threatening contagion infections in a multifariousness of patients population (Zeb *et al.*, 2017).The Gram-negative bacteria possesses linked outer membrane proteins (OPRs), which includes *oprI, oprL*, and *oprF* which attach it to the peptidoglycan and stabilizes the outer membrane (Beveridge, 1999). *P. aeruginosa* has been reported to possess *oprI,* a small outer membrane peptidoglycan-binding lipoprotein about 8-kDa in size (Duchene *et al.*, 1989; Remans *et al.*, 2010). The *oprI* is involved in envelope integrity via interactions

with peptidoglycan and other outer membrane proteins, majorly *oprF* and *oprL* (Chevalier *et al.*, 2017). which has also been reported to influence membrane vesicle development in *P. aeruginosa* (Wessel *et al.*, 2013).

Peptidoglycan-associated lipoprotein (*oprL*) and Outer membrane lipoprotein I (*oprI*) are peptidoglycan (secretory pumps) linked to the outer membrane protein in *P. aeruginosa*. The two genes are responsible for the intrinsic resistance to the antibiotics in *P. aeruginosa* (Sherman *et al.*, 2001; Priebe and Goldberg, 2014).

oprL and *oprI* lipoproteins are two outer membrane proteins of *P. aeruginosa*, and are responsible for the inherent resistance of the bacterium to antibiotics and antiseptics. As these proteins are found only in this organism, they could be used as a reliable marker for the rapid identification of *P. aeruginosa* in clinical samples (Osayande, 2010; Douraghi *et al.*, 2014). *oprL* mass (17.898 Da) is Part of the Tol-Pal system has an important role in the outer membrane invagination during the cell division and is significant for preserving the outer membrane integrity. Tol-Pal system consists of five core proteins are inner membrane proteins (*TolA, TolQ*, and *TolR)*, periplasmic protein TolB and outer membrane protein Pal. These proteins form a network linked the outer and inner membranes and the peptidoglycan (Fadhel and Hamim, 2020). With regard to oprI has mass (8.835 Da) (Neamah, 2017). Also, Alginate as an exopolysaccharide synthesized by *P. aeruginosa* plays an important role in development, maintenance, and spread of *P. aeruginosa* biofilms, encoded by a group of related genes including algD. *OprL* and *oprI* are outer bacterial membrane lipoprotein I and peptidoglycan-associated lipoprotein (*oprL*) that can be used as biomarkers for detecting *P. aeruginosa* in a bacterial isolated population (Mokhtari and Amini, 2019).

1.13.2. *LasR* and *LasI* Genes

These bacteria use intercellular communication, also called Quorum Sensing (QS), to regulate virulence and to acquire protection from environmental stress (Sharma *et al*., 2014). This process is coordinated through signalling molecules, known as acyl-homoserine lactones (AHL) that increase with cell density (Nazzaro *et al*., 2013). These molecules are produced by *lasI* synthase, reaching a threshold concentration sensed by the *lasR* protein triggering the activation or repression of specific genes related to virulence factors (Reading and Sperandio, 2006; Bottomley *et al.*, 2007). For this reason, this pathway is considered a potential target to control virulence of *P. aeruginosa.*

Quorum sensing (QS) is a bacterial cell–cell communication process that involves the production, detection, and response to extracellular signaling molecules called auto inducers (AIs), Quorum-sensing systems (QS) regulate the formation of Biofilm, in *P. aeruginosa*, play a key role in the differentiation process which increases concentration depending on density of the cells (Verhoeff *et al.*, 2006). Auto inducers (AIs) accumulate in the environment as the bacterial population density increases, and bacteria monitor this information to track changes in their cell numbers and collectively alter gene expression. QS controls genes that direct activities that are beneficial when performed by groups of bacteria acting in synchrony. Processes controlled by QS include bioluminescence, sporulation, competence, antibiotic production, biofilm formation, and virulence factor secretion (Pérez-Velázquez *et al*., 2016). *Pseudomonas aeruginosa* employs three major interconnected QS systems that function independently and dependently involving *las, rhl, pqs* pathways as well as novel candidate iqs pathway regulated by several QS signal molecules (Malešević *et al*., 2019). And these the three distinct systems are *las, rhl* and *mvfR* (*pqsR*) mediated by small diffusible signaling molecules called auto inducers (AIs): 4-hydroxy- 2-alkylquinolines (HAQs) and the N-acyl homoserine

lactones (AHLs): N-(3-oxododecanoyl)-L-homoserine lactone (3-OH-C12-HSL) and N-butanoyl-L-homoserine lactone (C4-HSL). The rhl system including rhlR (transcriptional regulator) and rhlI (autoinducer synthase) that precipitate in the synthesis of N-butyryl-L-homoserine lactone (PAI- 2). The second line consists of the las system (*lasR* and *lasI*) that produce N-(3-oxododecanoyl)-L-homoserine lactone (PAI-1) (El-Mowafy *et al.*, 2014). Figure (1-2). shows the three major *Pseudomonas aeruginosa* QS systems with their main effects.

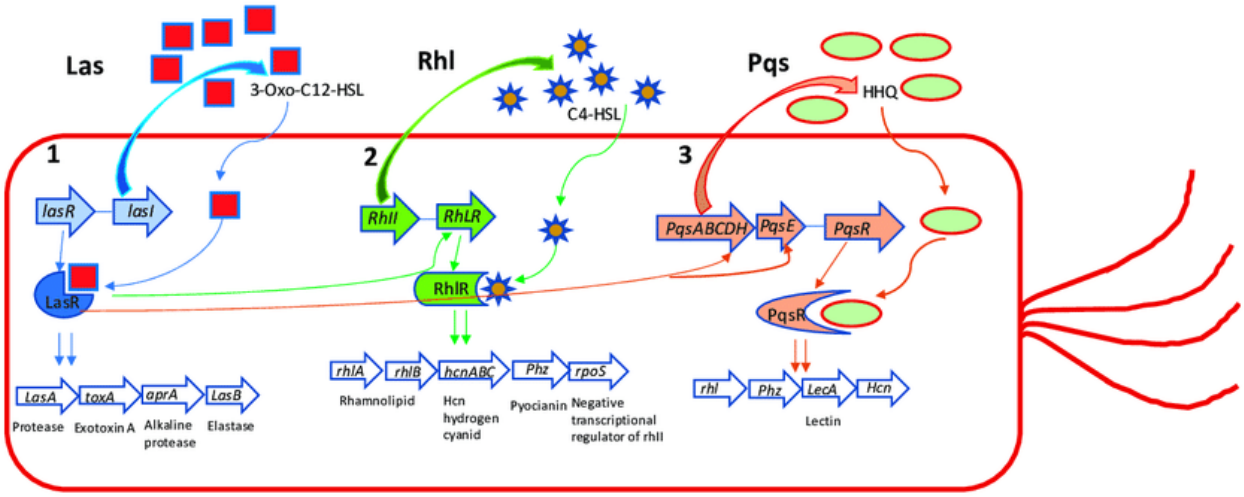


Figure (1-2). The three major *P. aeruginosa* QS systems with their main effects. (1) *LasI* produces 3-oxo-C12-HSL, which acts on *lasR*. This leads to induction of *aprA*, *lasA*, *las B*, and *toxA* genes and other virulence genes that are under its regulation. (2) *RhlI* produces C4-HSL, that acts on *RhlR*, which induces *phz*, *lasA*, *rpoS*, *lasB*, *rhlAB*, and *hcnABC* gene expression. (3) *PqsABCDH* produces HHQ that acts on *PqsR*, regulating the gene expression of *LecA*, *Phz*, *Hcn*, and *rhl*. Additionally, *lasR* positively (Guzzo *et al.*, 2020).

These signaling compounds are involved in the production of exoenzymes and regulation of virulence factors, bacterial as such, quorum sensing inhibitors (QSIs) diminish *P. aeruginosa* pathogenesis and microbial virulence and could have a major impact on the control and treatment of a wide range of acute and persistent infections (Kalia, 2013; Starkey *et al.*, 2014). Also, in *P.aeruginosa*, one of the main regulators of QS is the *LasR* protein, which needs, in order to regulate QS, to bind to its auto inducer, the molecule N-(3 oxododecanoyl)-homoserine lactone (OdDHL) (Huang *et al.*, 2016). The *lasR* gene codes the

transcription factor, which is responsible for the activation of numerous target genes, most of them related to QS in *P. aeruginosa*. *lasR* mutations are diverse as well as the phenotypes generated by them (Wang *et al.*, 2016). Moreover they also found that the gene *lasI* is required for synthesis of PAI, and that this molecule is responsible for the activation of *lasR* resulting in increased virulence factor transcription (Cooke and Schertzer, 2019). (Figure 1-3 and 1-4).

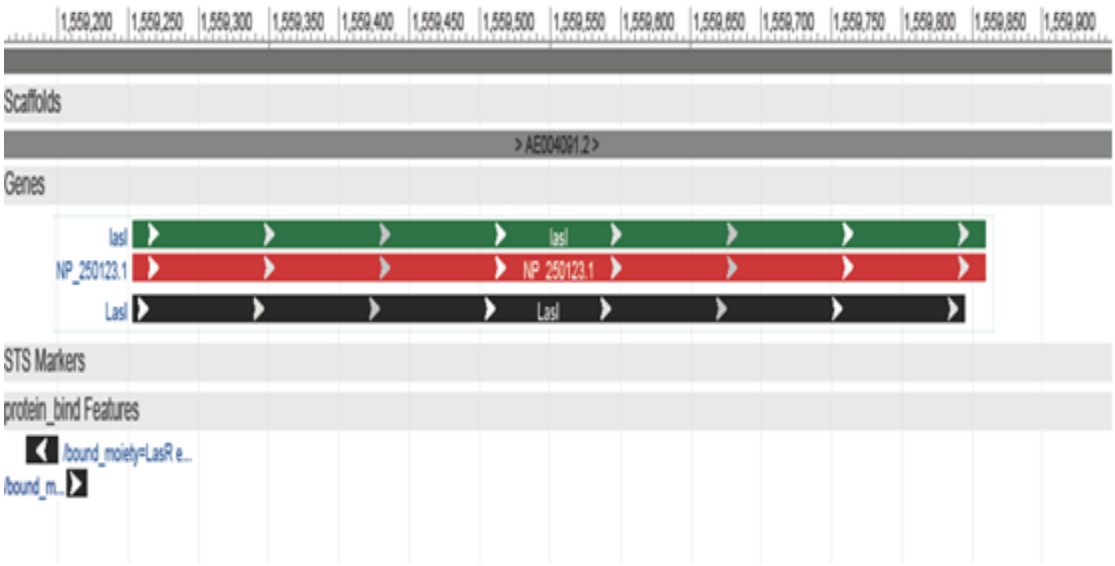


Figure (1-3). *LasI* in *P. aeruginosa* (NCBI).

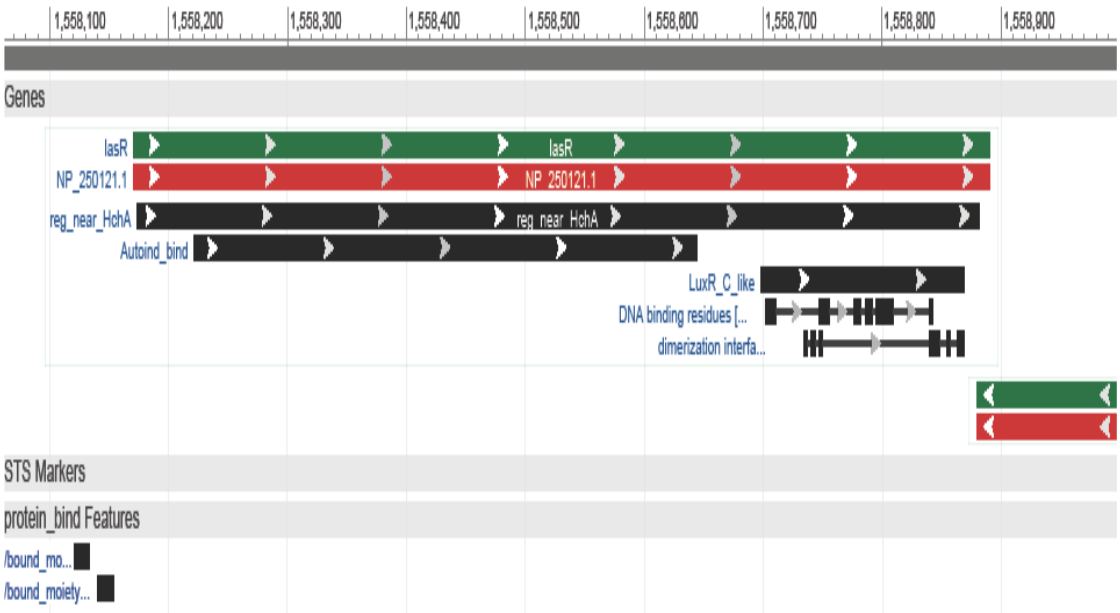


Figure (1-4). *LasR* in *P. aeruginosa* (NCBI).

1.13.3. The regulation of QS in *P. aeruginosa*

The regulation of QS in *P. aeruginosa* considering that the QS system plays an important role in controlling the bacterial genes, therefore, it is important to control and regulate its function. This regulation can be done as follows: The regulation of the transcription of *lasI* and *lasR* genes that induces changes in the amount of the production of synthase and R protein. The regulation by the molecular analogs of autoinducers produced by other bacteria or eukaryotic cells. The regulation by the molecules that can compete with R protein as Agonist or Antagonist. Enzymatic destruction like AHL degradation enzymes. In *P. aeruginosa*, *salR, vatM, posR, smaR, ponR* genes are the suppressor of the expression of *lasR* and *lasI* genes in the low density cells. The inactivation of anti-activator protein QScR encoded by *QScR* gene is triggered by interacting with *lasR* protein (Moghaddam, 2014). Figure (1-5).

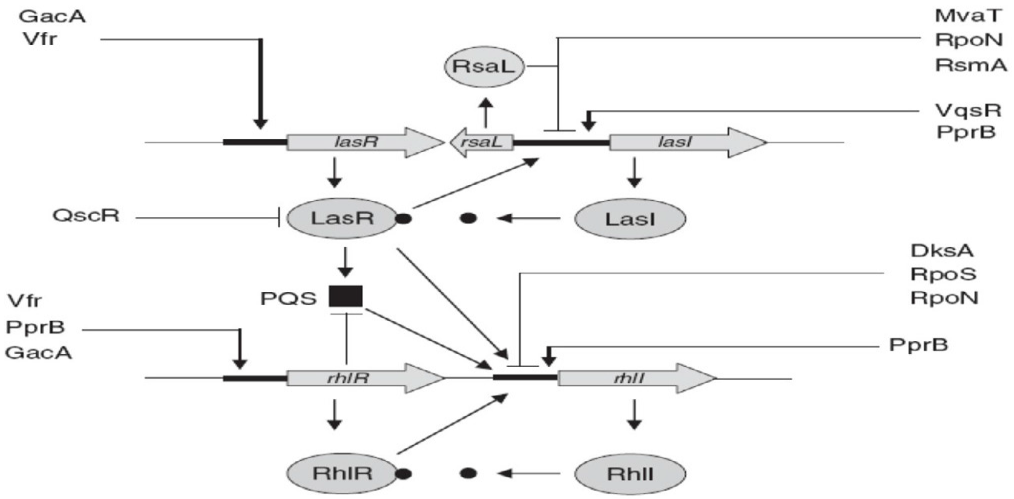


Figure (1-5). The process of the external regulation of genes in *P. aeruginosa* QS system (Moghaddam, 2014).

1.14. PCR and DNA sequencing of genes *lasI* , *lasR* ,*oprI* and *oprL*

The polymerase chain reaction (PCR) discovered in 1985 revolutionized the diagnosis of infectious diseases in clinical laboratories by allowing rapid, sensitive and specific detection and identification of pathogens directly from

clinical specimens, without the need for culture. PCR-based assays enable the amplification of a few target molecules (theoretically a single cell) to detectable levels, from both viable and non-viable cells. PCR (Polymerase Chain Reaction) is one of the most powerful analytical techniques ever developed (Ochsner *et al*., 2002).

Sequencing is the process of determining the precise order of nucleotides within a DNA molecule. The advent of rapid DNA sequencing methods has greatly accelerated biological and medical research and discovery (Ehsani *et al.*, 2016). Knowledge of DNA sequences has become indispensable for basic biological research, and in numerous applied fields such as medical diagnosis, biotechnology, forensic biology, virology and biological systematics. Comparing healthy and mutated DNA sequences can diagnose different diseases including various cancers (Chmielecki and Meyerson, 2014). characterize antibody repertoire, and can be used to guide patient treatment. Having a quick way to sequence DNA allows for faster and more individualized medical care to be administered, and for more organisms to be identified and cataloged. The rapid speed of sequencing attained with modern DNA sequencing technology has been instrumental in the sequencing of complete DNA sequences, or genomes, of numerous types and species of life, including the human genome and other complete DNA sequences of many animal, plant, and microbial species (Abate *et al.*, 2013).

1.15. High-throughput whole genome sequencing

All genetic elements and factors pertaining to antimicrobial drug resistance must be identified via molecular tests (De Bentzmann and Plésiat, 2011). In addition to antibiotic resistance control and surveillance, the high-throughput whole genome sequencing (WGS) approach is useful in analyzing the potential adaptation and evolution of *P. aeruginosa* over time (Snyder *et al.*, 2013; Köser *et al*., 2014). Before the discovery of the whole genome sequencing (WGS)

approach information on bacterial phylogeny was limited, and relationships among different pathogens were lacking. The emergence of high-throughput WGS along with bioinformatics techniques such as multi-locus sequence typing (MLST) allows the construction of phylogenetic trees and identification of the genetic structures of bacterial species (Klemm and Dougan, 2016). High-throughput WGS has also proved to be useful in the development of antimicrobial drugs and vaccines. By combining WGS with SNP-based analysis, novel antibiotic targets can be determined by comparing the SNPS in the genomes of a sensitive bacterial parent strain and a resistant bacterial daughter strain (Loman and Pallen, 2015). High-throughput WGS also drove the development of SNP-based phylogenetic analysis which proved to be useful in determining the relationship between genetic diversity and different niches for pathogenic bacteria, hence uncovering the epidemiology of outbreaks in hospital settings. The feasibility of these technological advances has facilitated the development of transmission mapping while identifying pathogen diversity within the host. This means that WGS has allowed microbiologists to monitor bacterial evolution and investigate the spread of bacterial pathogens at different scales (globally, locally and within a single patient).It can also allow the identification of previously unknown microorganisms, virulence factors and antibiotic resistance determinants (Robinson *et al*., 2013).

1.16. Cytokines

Low molecular weight protein is released by cells that have a specific effect on the interactions between cells of the immune system in response to infection. The cytokines include interleukins, lymphokines, tumor necrosis factors, interferons, chemokines and monokines. Interleukins are the cytokines made by one leukocyte and acting on other leukocytes (Sprague and Khalil, 2009).

Major burn injury induces an inflammatory response that is characterized by the activation of inflammatory pathways and the release of various cytokines .

Inflammation is controlled by the balance between pro- and anti-inflammatory mediators in a complex cytokine network. In this network, one cytokine can influence multiple cell types (pleiotropy), and multiple cytokines can have similar biological effects on the same cell type (redundancy). Hence, the action of one cytokine can be compensated by another cytokine.

1.16.1. Interleukin-8

Interleukin-8 is a chemokine which belongs to Cysteine X Cysteine (CXC) family with the amino acid sequence Glu-Leu-Arg (ELR) preceding the first conserved cysteine amino acid residue in its primary structure and serves as a chemical signal that attracts neutrophils to the site of inflammation. IL-8 is involved in the progression of severe sepsis and it has been reported that serum and plasma levels of IL-8 were enhanced in patients with sepsis (Miyoshi *et al.*, 2010 ; Macdonald *et al.*, 2014). Furthermore Interleukin-8 is a chemoattractant cytokine for neutrophil and is associated with multiple organ failure. IL-8, also known as CXCL-8, is one of the significant chemokines for leukocyte recruitment expressed by various cells such as macrophages/monocytes, endothelial cells, fibroblasts, epithelial cells, and neutrophils (Wan Yusoff *et al.*, 2020). IL-8 is a member of the family of chemokine which is known for its leukocyte and lymphocyte chemotactic properties and plays vital role in the initiation and amplification of acute inflammatory reaction and in the chronic inflammatory process as a pre-inflammatory cytokine (Hu *et al.*, 2016).

Interleukin-8 is secreted by malignant cells and tumor stroma cells across many different tumor types (Brahmer *et al.*, 2012; Hamid *et al.*, 2013). Moreover the serum IL-8 is a promising biomarker for CRC detection and may represent a clinically useful tool to identify high-risk patients.(Jin *et al.*, 2014). IL-8 affects the function of several cell types, notably neutrophils, causing chemotaxis, adhesion, and activation and acts on endothelial cells leading to angiogenesis. It mediates shedding of L-selectin and rapid up-regulation of a variety of adhesion

molecules (CR1, CD11b/ CD18, CD11c/CD18) on the neutrophil surface, facilitating their adherence to endothelial cells. In addition, IL-8 causes remodeling of the neutrophil cytoskeleton and chemotaxis along the IL-8 concentration gradient originating from the site of inflammation (Williams *et al.*, 1996). Interleukin-8 (IL-8), also contributes significantly to SP (neuropeptide substance P) enhanced keratocyte migration and is able to attract neutrophils. In addition, the preferred SP receptor, the neurokinin-1 receptor, is necessary to induce keratocyte migration and IL-8 secretion (Sloniecka *et al.*, 2016).

Interleukin-8 was first characterized in 1987. Since then, knowledge regarding its role in leucocyte trafficking and activation has advanced rapidly, especially in the field of cardiovascular disease. In the scientific literature, there is sufficient evidence to support beyond any doubt the involvement of IL-8 in the establishment and preservation of the inflammatory micro environment of the insulted vascular wall (Apostolakis *et al.*, 2009).

1.16.1.1. Interleukin-8: Structural and functional characteristics

Interleukin-8, or CXCL8 based on the latest nomenclature, represents the prototypical chemokine of the CXC subfamily (Remick, 2005). IL-8 is actively secreted in the extracellular space as a result of a variety of cellular stimuli. It is a small protein; its mature, fully active form has only 72 amino acids. Transcription of the IL-8 gene encodes for a protein of 99 amino acids that is proteolytically cleaved to a biologically active peptide of either 77 amino acids in non-immune cells or 72 amino acids in monocytes and macrophages (Waugh and Wilson, 2008). A wide variety of cell types, including virtually all nucleated cells, are potential sources of IL-8. However, the principal cellular sources of IL-8 are typically monocytes and macrophages. IL-8 bears the primary responsibility for the recruitment of monocytes and neutrophils, the signature cells of acute inflammatory response. Cellular recruitment occurs through the

development of a chemotactic gradient, which causes the inflammatory cell to move towards an area of increased chemokine concentration.

In vivo, the chemotactic gradient may be generated by the binding of IL-8 to basement membrane proteins. This gradient aids in bringing cells towards the site of inflammation and also retains them once they have arrived. In addition to recruitment, IL-8 serves to promote the activation of monocytes and neutrophils (Remick, 2005 ; Apostolakis *et al.*, 2009). The biological effects of IL-8 are mediated through the binding of IL-8 to two cell surface receptors, CXCR1 and CXCR2. These G-protein-coupled receptors share considerable structural similarity and induce a nearly identical range of biological activities (Apostolakis *et al.*, 2009). Signals are transmitted across the membrane through ligand-induced structural changes, exposing epitopes on the intracellular loops and carboxy-terminal tail of the receptor. These epitopes promote coupling to functional heterotrimeric G proteins (Moser and Loetscher, 2001; Schraufstatter *et al*., 2001).

The biological activity of IL-8 and other CXC chemokines is in part dependent on the ELR amino acid motif. The presence of these three specific amino acids is crucial for the binding of IL-8 to its receptor. IL-8 is resistant to temperature and proteolysis, and is relatively resistant to acidic environments. These biochemical characteristics make it an ideal candidate molecule for sites of acute inflammation, where it must withstand harsh and hostile conditions.

Another unique functional characteristic of IL-8 is its relative longevity at sites of acute inflammation. It is produced early in the inflammatory response, but remains active for a prolonged period of time, even days and weeks. This is in contrast to most other inflammatory cytokines, which are typically made and cleared in vivo in a matter of hours. A third interesting aspect IL-8 involves the oxidant regulation of gene expression. IL-8 is highly sensitive to oxidants, and anti-oxidants substantially reduce IL-8 gene expression. The role of oxidants in

the regulation of IL-8 and other chemokines has relevance in the field of cardiovascular disease, where ischaemia induced oxidative stress is both a marker of disease and a potential therapeutic target (Apostolakis *et al.,* 2009).

1.16.1.2.Pathophysiological implications of interleukin-8

Interleukin-8 is an important inflammatory mediator. It is produced in a variety of tissues upon exposure to inflammatory factors, such as tumor necrosis factor (TNF) or IFN. Upon release, it recruits lymphocytes and activates neutrophil granulocytes, which in turn can produce IL-8 themselves, amplifying the inflammatory signal. IL-8 is degraded relatively slowly compared to other cytokines and can persist for several days in its active form in the direct environment of the cells from which it has been released. which makes it easier to measure in studies than other interleukins. IL-8 can also be produced by a variety of cells inside the CNS, including astrocytes, endothelial cells, and microglial cells, when stimulated by cytokines or other infectious stimuli (Kuzior *et al.,* 2020). CXCR2-receptors for IL-8 can be found on neurons and endothelial cells of the blood–brain barrier (BBB) (Stuart and Baune, 2014).

There is also evidence that IL-8 has neuromodulatory properties. Puma and colleagues were the first to show that IL-8 can directly modulate ion channels, specifically calcium channels, in the brain and reduce calcium currents, thus influencing synaptic activity. This observation indicates that IL-8, through the direct modulation of neuronal excitability, might be an important factor in intercellular communication under normal and pathological conditions (Puma *et al.,* 2001). In their recently published review, Stuart and colleagues suggested that IL-8 also affects the hypothalamus-hypophysis axis (Stuart *et al*., 2015). This stress axis plays an important role in the development and maintenance of various mental illnesses; and the hyperactivity of the hypothalamus-pituitary gland axis in depressive disorders is a well-known phenomenon (Otte *et al.*, 2016). From this perspective, upregulation of IL-8 might be a compensatory

response during disease/stress .In summary, IL-8 is a potent pro-inflammatory mediator that promotes the migration of neutrophil granulocytes and other pro-inflammatory cells into inflamed tissues; in addition, it can be secreted by microglia. Furthermore, IL-8 fulfills complex signaling functions. It is quite conceivable that in pathological states, IL-8 can intervene in and modulate various neuronal processes and central metabolic pathways (Semple *et al*., 2010).

1.17. Role of IL-8 in Burn Patients

The IL-8 play a crucial role in burn injury and the serum IL-8 level could be a biomarker for mortality in burn patients. Inflammatory reaction is caused by burn injury in the early hospital phase. Subsequently, leads invasion of microorganisms for burn wounds to exacerbates inflammation, cytokines release. The excessive release of inflammatory cytokines results in serious systemic inflammation that induces tissue damage and vascular endothelial injury and that can progress to multiple organ failure and eventually lead to the patient's death (Matsuura *et al*., 2019).

Chapter Two

Materials and Methods

2.1. Materials

2.1.1. Equipment and Instruments

The instruments and equipment which were used in this study were listed in the table (2-1).

Table (2-1). Laboratory Equipment and Instrume3nt that used in this study.

NO	Equipment/Instrument	Company	Origin
1	Autoclave	Stermite	Japan
2	Benson burner	Citotest	China
3	Centrifuge	Hettich	Germany
4	Cooled centrifuge	Eppendorf 5417R	Germany
5	DNA extraction tubes 100 µl. P.C.R tubes 50µl.	Capp	Germany
6	Electrophoresis constant power	Biocom detection	Indian
7	ELISA plate	Elabscience	Korea
8	ELISA Reader	Biotech	United State
9	Eppendorf rack		China
10	Freezer	Aucma	
11	Gel electrophoresis system	Clever	United State
12	Gel tube	Afco	Jordan
13	Glass wares (beaker, rounded flask, cylender)	Slamid U.K Hysil	England
14	Hood	Gallenkamp	
15	Incubator, Oven	Memmert	Germany
16	Laboratory Distillation Unit	GFL	Germany
17	Micropipettes 5-50 µl ,100-1000 µl, 0.5-10 µl , Multichannel pipette	Capp	Germany
18	Microwave	LG	Thailand
19	Multichannel pipette	Slamed	Japan
20	Nano Drop	Implen	Germany
21	Para film	Bemis	Korea
22	PCR Thermal Cycler	Clever	United State
23	PCR tubes 1.5 ml Eppendorf	Biobasic	Canada
24	PCR tubes 200µl Eppendorf	Biobasic	
25	Petri dishes 9 cm	Afco	Jordan
26	Plain tube 10 ml	Afco	
27	Loop	Himedia	Indian
28	Premium quality Petri dishes divided	Afco	Jordan
29	Rack	HDA	China
30	Refrigerator	Concord	Italy
31	Sensitive Balance	Denver	United State
32	Sequenced by Genetic Analyzer system (ABI-310)	Macrogen	Korea
33	Sterile swab	Afco-Dispo	Jordan
34	Tips	Sterellin Ltd.	UK
35	Transport collection swabs	Citotest LaB ware	China
36	UV-trans illuminator	Clever	United State
37	VITEK 2- Compact	Biomerieux	France

38	Volumetric Cylender	HDA	China
39	Vortex	Clever	United State
40	Wooden sticks	Supreme	China

2.1.2. Chemical Materials

Chemical materials used in this study were listed in table (2-2).

Table (2-2). Chemical materials that used in this study.

No.	Chemical Materials	Company/origin
1	Agarose	Promega/ USA
2	Alcohol 70%	Fluka chemical
3	Deionized Distilled water (ddH_2O)	Bioneer / Korea
4	DNA ladder 100bp	Promega/ USA
5	Ethanol (C2H5OH) 95%	BDH/ England
6	Ethidium Bromide	Bioneer / Korea
7	Glycerol (C3H8O3)	PanRech / Spain
8	Loading dye (bromophenole blue)	Promega/ USA
9	Master mix	
10	Phosphate buffered saline	Bio basic / Canada
11	Sterile Normal Saline	Bioneer / Korea
12	Tris-Borate-EDTA (TBE) buffer	Promega/ USA
13	Tris-EDTA buffer (TE)	

2.1.3. Biological Materials

Biological materials that used in this study were listed in table (2-3).

Table (2-3). Culture media that used in this study.

NO.	Culture media	Company/origin
1	Blood Agar Base	Condalab / Spain
2	Brain Heart Infusion broth	Oxoid /United Kingdom
3	MacConkey Agar	Condalab / Spain
4	Nutrient agar	
5	Pseudomonas chromogenic agar	

2.1.4. Diagnostic Kits

Table (2-4). List of diagnosis kits and molecular material used during this study.

Table (2-4). The molecular material and diagnostic kits that used in this study.

NO.	Types of kits	Company/ Country
1	$VITEK_2$ GN Gram-Negative Identification	Biomerieux / France
2	VITEK2 AST-GN76	
3	Genomic DNA Extrection Kit (G-Spin)	iNtRon/Korea
4	DNA ladder 100bp	Promega/ USA
5	Master mix	Promega/ USA
6	Human IL-8 ELIZA Kit	Elabscience / U.S.A

2.1.5. Antibiotics susceptibility

Table (2-5) shows a list of Antibiotics to the contents of the AST-GN76 card used during this study for susceptibility testing by the Vitik2 system.

Table (2-5). Content Antibiotic cards for AST-GN76 that used in this study by Vitek2 compact according to the manufactures company (Biomerieux, France).

Antimicrobial	Code	Concentration µg/ml	Calling Range	
			≤	≥
Amikacin	AN	8, 16, 64	2	64
Ampicillin	AM	4, 8, 32	2	32
Cefazolin	CZ	4, 16, 64	4	64
Cefepime	FEP	2, 8, 16, 32	1	64
Cefoxitin	FOX	8, 16, 32	4	64
Ceftazidime	CAZ	1, 2, 8, 32	1	64
Ceftriaxone	CRO	1, 2, 8, 32	1	64
Ciprofloxacin	CIP	0.5, 2, 4	0.25	4
Ertapenem	ETP	0.5, 1, 6	0.5	8
ESBL	ESB	FEP 1, CTX 0.5, FEP/CA 1/10, CTX / CA 0.5/4, CA/CA 0.5/4	NEG	POS
Gentamicin	GM	4, 16, 32	1	16
Imipenem	IPM	1, 2, 6, 12	0.25	16
Levofloxacin	LEV	0.25, 0.5, 2, 8	0.12	8
Nitrofurantion	FT	16, 32, 64	16	512
Piperacillin/ Tazobactem	TZP	2/4, 8/4, 24/4, 32/4, 32/8,48/8	4/4	128/4
Tigecycline	TGC	0.75, 2, 4	0.5	8
Trimethoprim/ Sulfamethoxazole	SXT	1/19, 4/76, 16/304	20 (1/19)	320 (16/304)

2.1.6. Primers Used for the Detection of *P. aeruginosa*

Primers that used in this study were listed in table (2-6).

Table (2-6). Primers that used in this study.

Priner Name	Sequence 5´ to 2´	Annealing Temp°C	Product size pb	Reference
LasI	F 5´-ATGATCGTACAAATTGGTCGG-3´ R 5´-GTCATGAAACCGCCAGTC-3´	57	600	(Aghamollaei *et al*., 2015)
LasR	F 5´-ATGGCCTTGGTTGACGGT-3´ R 5´-GCAAGATCAGAGAGTAATAAGACCC-3´	57	700	(Aghamollaei *et al*., 2015)
OprI	F 5´-ATGAACAACGTTCTGAAATTCTCTGCT-3´ R 5´-CTTGCGGCTGGCTTTTTCCAG-3´	57	249	(Jami Al-Ahmadi and Zahmatkesh Roodsari, 2016)
OprL	F 5´-ATGGAAATGCTGAAATTCGGC-3´ R 5´-CTTCTTCAGCTCGACGCGACG-3´	57	504	(Jami Al-Ahmadi and Zahmatkesh Roodsari, 2016)

2.1.7. The content of DNA extraction kit G-spin/ Korea that used in this study

Ethanol alcohol (96-100%), Proteinase K solution for precipitation proteins, G buffer, Binding buffer, Washing buffer A, Washing buffer B, Elution buffer, RNase A solution and Lysozyme solution.

2.1.8. Master Mix

Table (2-7). The contents of master mix that used in this study.

NO.	Materials
1	DNA polymerase enzyme (Taq)
2	dNTPs (400µm dATP, 400µm d GTP, 400µm dCTP, 400µm dTTP)
3	MgCl2 (3mM)
4	Reaction buffer (pH 8.3)

2.1.9. DNA ladder

Table (2-8). The ladder and loading dye composed that used in this study.

NO.	Materials
1	Ladder consists of 11 double-stranded DNA with size 100-1500bp
2	Loading Dye has a composition (15% Ficoll, 0.03% bromophenol blue, 0.03% xylene cyanol, 0.4% orange G, 10mM Tris-HCl (pH 7.5) and 50mM EDTA)

2.1.10. Human Interleukin-8 ELIZA Kit

The Eliza kit was used for detection and the levels of Interleukin-8 in this study compact according to the manufactures company (Elabscience / U.S.A) No.E-EL-H6008.

2.2. Methods

2.2.1. Study Design of *Pseudomonas aeruginosa*

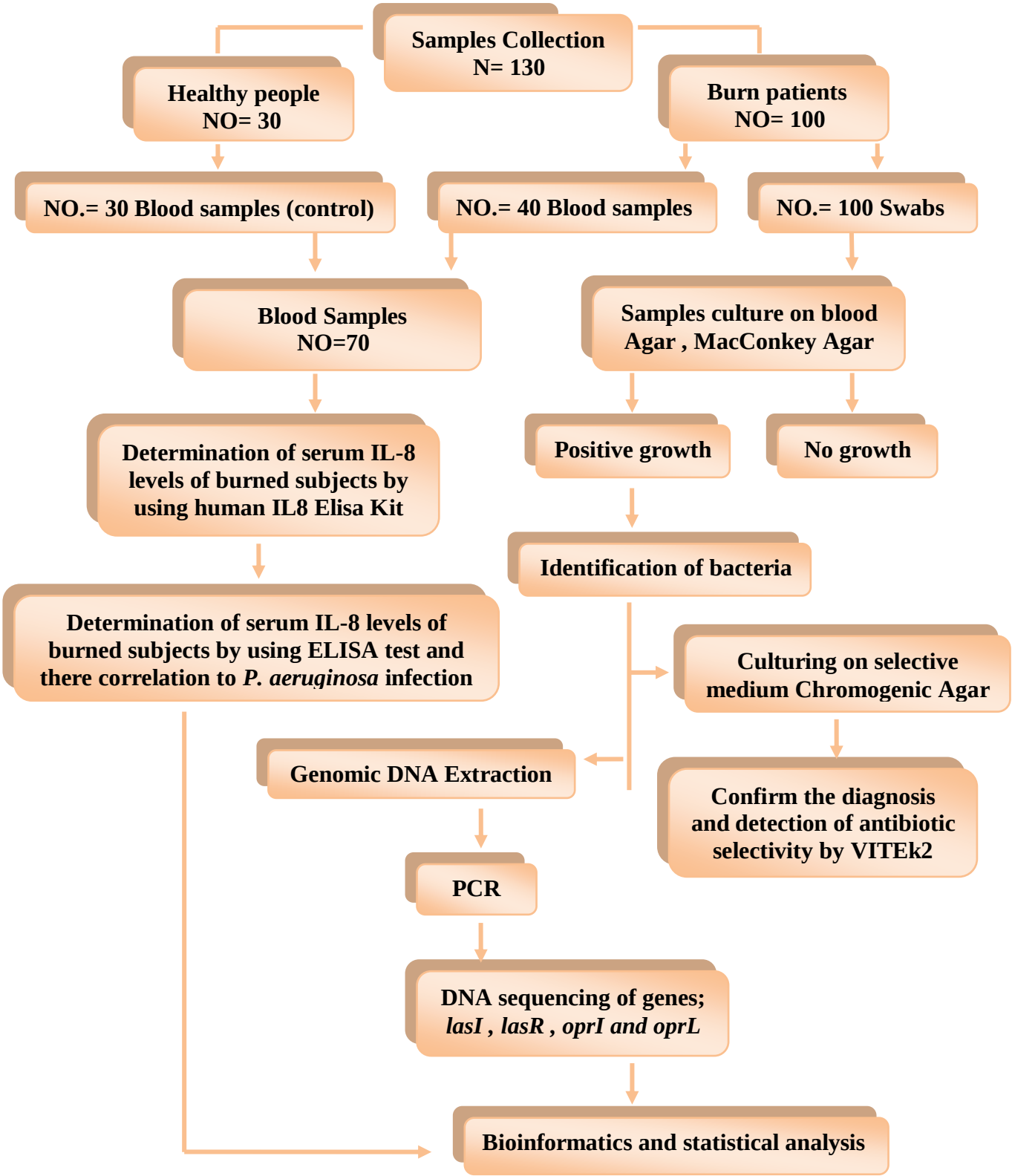


Figure (2-1). Experimental design of the study.

2.2.2. patients and Sample Collection

A total of 130 samples (100 swabs and 40 blood sample from the same burned patients and 30 blood sample from healthy individuals) were collected from three different hospitals in three governorates. The burn unit at Imam Al-Sadiq Hospital (peace be upon him) in Babylon, the burn center at Al-Hussein Teaching Hospital in Kerbala and the burn center in Al-Sadr Medical City in Najaf during the period from December 2020 to April 2021,which include 100 burned patient, from these 100 swabs were collected. For blood samples, 40 blood samples were collected from 40 patients and 30 blood samples were collected from healthy individuals from Babylon used as control for IL8 detection.

Patients ages ranged from 1 year to 80 years, males were 46 patient (14 adult, 32 children) and 54 patients were females (40 adult ,14 children) they were suffered from second to third degree burns by means of (flame, hot fluids, gasoline, oil, electricity). The burn rate ranged between (5-100%) of the total body surface area (TBSA). All patients were diagnosed by a consultant physician.

2.2.3. Ethical Approval

A valid consent was achieved from hospital administration and from each (patients) before their including in the study. For every patient or their followers, the procedure had been informed before the samples were collected, making absolutely sure that they understood the procedure that was to be carried out. The patients had the right to reject giving the sample and including in the study.

2.2.4. Sample processing

This study was conducted in the laboratory of Imam Al-Sadiq Hospital (peace be upon him) and the DNA laboratory at the University Babylon / College of Science.

2.2.4.1. Burn Swabs

One hundred swab samples were taken from burn patients , after three and six days of burn patients from the pus of the burned area in the morning before the bathing of the affected area (before hydrotherapy) by transport and collection swabs (sterile swab stick, sterile transport medium swabs). Each swab was placed in a sterile tube till reaching the laboratory to be inoculated on culture media (Blood agar, MacConkey agar) and all gram negative bacteria that's grow on MacConkey agar were cultured on Pseudomonas chromogenic agar and incubated aerobically for (24-48) hours at 37C˚ (Collee *et al.*, 1996). The conformational diagnosis of *P. aeruginosa* and antibiotic sensitivity test were confirmed by using Vitek2 compact device.

2.2.4.2. Blood Sample

Forty blood samples were taken from burned patients: 5 ml after (3-6) days from burn injury in addition to 5 ml blood sample were taken from 30 healthy people as a control group used to determine the level of cytokines and placed in a sterile gel tube. The samples are centrifuged at 1500 cycles for 5 minutes and the serum is stored at -20 °C until use in analysis (Lewis *et al.*, 2001)

2.2.5. Preparation of culture media

Nutrient agar, MacConkey agar, Brain heart infusion broth , Pseudomonas Chromogenic Agar, Muller Hinton agar , Blood Agar Base were prepared according to the information described on the containers and sterilized by the autoclave 121°C for 15 min under 1.5 bar and to ensure that these media were not contaminated, sterility test was performed by putting them in the incubator at (37°C) for (24 hrs.), if there is no growth in the media, plates were kept in the refrigerator.

2.2.5.1. Blood Agar Medium

This media were prepared according to manufacturing company (Condalab / Spain) instructions sterilized by autoclaving and cooled to 45°C then 5% of fresh human blood was added. This media were used for cultivation of most of the pathogenic bacteria and also used for examination of their ability to hemolyze RBCs and determination of the type of hemolysis. Positive result for *P. aeruginosa* in blood agar is typically displayed beta hemolysis, a metallic sheen, and blue or green pigment (Hogardt and Heesemann, 2010; Korgaonkar *et al.*, 2013).

2.2.5.2. MacConkey agar medium

MacConkey agar medium has been prepared according to the method described by the manufacturing company (Condalab / Spain) and it is used for the primary isolation of most Gram- negative bacteria This medium contains the crystal violet to prevent the growth of the Gram-positive and allow to grow the Gram-negative bacteria and differentiation of lactose fermenter from the non-lactose fermentative. *P. aeruginosa* appear non-lactose fermenter on the MacConkey agar (Allen, 2013).

2.2.5.3. Brain Heart Infusion broth

This medium was prepared according to the manufacturing company (Oxoid /United Kingdom) and it was used for activation of bacteria and for general experiments (MacFaddin, 2000).

2.2.5.4. Pseudomonas chromogenic agar

Pseudomonas chromogenic agar medium was prepared according to the method recommended by the manufacturing company (Condalab / Spain). Suspend 37 grams of the medium in one liter of distilled water previously heated to 80 ºC. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolvence. Pseudomonas Chromogenic Agar is selective

medium and useful for the identification of *P. aeruginosa* the medium can be inoculated directly with the loop. Incubate at 37 ºC for 24-48 hours. *Pseudomonas* spp is easily distinguished due to the magenta colony color and the color of the medium will change from green to blue-green. The other types of bacteria are inhibited, and in case of growing, they grow as colorless colonies.

2.2.5.5. Nutrient Agar medium

Nutrient agar medium has been prepared according to the manufacturing company. It has been used for general experiment isolate culture, cultivation and activation of bacterial isolates when it is necessary (MacFaddin, 2000).

2.2.6. Procedure of diagnosis of bacteria isolates by VITEK2-Compact

The identification of *Pseudomonas aeruginosa* was confirmed using VITEK2-Compact which represents an advanced colorimetric technology for bacterial identification. ID gram negative(GN) card was used for this purpose for *Pseudomonas aeruginosa* identification and used gram negative susceptibility card (AST-GN76) for susceptibility testing against specified antimicrobials.

Procedure

All the following steps were done according to the manufacturing company (Biomerieux, France).

1- The suspension was prepared from a new culture at 24 hours at a temperature of 37˚C, then 3mL of physiological salt solution was placed in sterile tubes, then 3 colonies or less of bacteria were transferred to each tube.

2- The colonies were mixed by the Auto Vortex device.

3- The turbidity of the suspension was standardized by Densichek at a rate of 0.5 - 0.65 with a McFarland standard solution.

4- Appropriate card was attached to the cassette.

5- the strip code of the card was read and matched with commentary card code.

6- Information has been recorded regarding the name and sequence of the sample.

7- The cassette was putted in the specified location of the device.

8- After the cards are filled out, the user records the cassette to the automatic reader incubator and the cards are checked automatically by the internal barcode reader, then plugged and loaded into the internal reader incubator. Then system automatically ejected the cards into a waste container.

9- The card was analyzed using the VITEC system after 4-8 hours, and the report was obtained, which is stored in the device's memory.

2.2.7. Sterilization methods

According to the method described by Rutala and Weber (2001) with slight modification.

2.2.7.1. Dry Heat Sterilization

Electric oven was used to sterilize glass wares and others at 160 °C for 2-3 hrs.

2.2.7.2. Autoclaving

Media and solutions were sterilized by autoclaving at 121°C for 15 min under 15 psi pressure.

2.2.8. Maintenance of Bacterial isolates

Maintenance of isolates was performed according to Vandepitte *et al.,* (2003), and as follows

2.2.8.1. Daily Working Culture

For the purpose of daily use of bacterial cultures, the bacterial isolates were inoculated in the culture media, which were incubated at a temperature of 37 °C for 24 hours, and then kept in the refrigerator until its use.

2.2.8.2. Long-term isolates

For the purpose of preserving the isolates for a long time without the possibility of them losing some of their genetic characteristics, a brain-heart infusion broth was used with 15% glycerol, as a test tube containing 5-10 ml of

the medium was inserted into one colony. The culture was incubated for 24 hours, then 0.85 ml of the culture was transferred to vials with a tight cap containing 0.15 ml of sterile glycerol. The mixture was mixed by turning the tube down and up after closing it several times and the culture was stored at -20 °C until use and the farms were renewed every three months.

2.2.9. Preparation of Solutions

2.2.9.1. Normal Saline

It was prepared by dissolving 0.85g of NaCl in 90 ml distilled water and further completed to 100 ml with D.W (Collee *et al.*, 1996).

2.2.9.2. TBE Buffer (Tris-Borate-EDTA)

TBE running buffer is the most commonly used buffer for DNA and RNA gel electrophoresis. TBE was prepared and stored as a 10× stock solution. The 10× working solution was prepared by dissolving 108 g of Tris base, 55 g of boric acid, and 40 ml of 0.5 M EDTA in 1000 ml of D.W. However, final concentration of 1×TBE solution was prepared by adding 100 ml of 10× TBE buffer to 900 ml of sterile D.W (Sambrook and Russell, 2001).

2.2.9.3. Tris-EDTA Buffer (TE buffer)

This buffer was prepared by mixing ten µl Tris-HCl and one µl, EDTA-Na_2 with distilled water. Then, the pH was adjusted to 8.0. It was sterilized by autoclaving at 121 °C for 15 minutes and stored at 4 °C (Soini and Musser, 2001).

2.2.9.4. Loading Buffer

It was prepared by mixing 0.25% bromophenol blue and 40% sucrose using distilled water. It was stored in a dark container at 4 °C (Soini and Musser, 2001).

2.2.9.5. Phosphate Buffered Saline

It was prepared by dissolving 8.0gm NaCl, 0.2gm KCl, 1.44gm Na_2HPO_4, 0.24 gm KH_2PO_4 in 1000 ml of distilled water and sterilized by autoclaving at 121 °C for 15 minutes then stored at 4 °C (Soini and Musser, 2001).

2.2.9.6. Ethidium bromide solution

It was prepared by dissolving 0.05gm of ethidium bromide in 10ml distilled water and stored in dark reagent bottle (Sambrook and Russell, 2001).

2.2.10. Molecular Diagnosis

2.2.10.1. Solutions Used in DNA Isolation

Loading Buffer, Tris-EDTA Buffer (TE buffer), Phosphate Buffered Saline, Ethidium bromide solution and Tris-borate-EDTA Buffer (TBE).

2.2.11. DNA extraction

The total genomic DNA were extracted using G-spinTM Genomic DNA Extraction Kit [for Bacteria] (Cat. No.: 17121) according to the manufacturer instruction (Intron Bio/Korea). All samples electrophoresed using 0.7% agarose gel to confirm the extracted DNA (Sambrook and Russell, 2006; Dong *etal*., 2019).

2.2.11.1. DNA extraction (Gram-Negative) bacteria protocol.

1- The bacterial culture broth grown in nutrient broth media in (Plain tube 10 ml) transport to centrifuge device for 3 min at 6000 rpm and then the supernatant discarded.

2- Cell Harvesting: the bacterial culture was transferred to a 1.5 ml micro centrifuge tubes.

3- Then centrifuged for 1 min. at 13.000 rpm and the supernatant was then discarded.

4- Three hundred µl of cell lysis G-Buffer was added to the sample and mixed by vortex.

5- Then incubated at 65°C for 15 minutes or until the sample lysate is clear. During incubation, the tubes were inverted for every 5 minutes.

6- A 250 µl of Binding buffer was added and completely mix well by pipetting (at least 10 tiems) or gentily vortexing.

7- cell lysates were loaded on column and centrifuged at 13.000 rpm for 1 min.

8- wash. 500 µl of washing buffer A was added to colum and centrifuged at 13.000 rpm for 1 min.

9- Remove solution. 500 µl of washing buffer B was added to colum and centrifuged at 13.000 rpm for 1 min.

10- solution was removed and centrifuged at 13.000 rpm for 1 min.

11- G-spin column was placed in a clean 1.5 ml micro centrifuge tube and 100 µl of elution buffer was added directly onto the membrane.

12- Then tube was incubated for 1 min at room temperature then centrifuge at 13.000 rpm for 1 min.

13- Tube then kept in the freezer at - 20°C.

2.2.12. Estimation of DNA Concentration and Purity

The concentration and purity of extracted DNA and estimated by using nano drop (Implen / Germany) the excepted ratio of pure DNA is between 1.8-2.

2.2.13. Protocol of Gel Electrophoresis

2.2.13.1.Preparation of Agarose Gel 1%

For detection of DNA after DNA extraction. 0.5 gm of agarose was boiled in 50 ml 1X TBE buffer, left to cool at 50°C and 2µl of Ethidium bromide was added to agarose and poured on preparing tray. The comb was removed after hardening of agarose leaving wells. Loading 5 µl DNA and two µl loading dye and making electrophoresis diagnose DNA in a sample of extracted. The power was set in the power supply usually at 60 voltages to resolve DNA under an electric field. After 60 minutes, the following electrophoresis, the products were visualized using UV light on a transilluminator and photographed (Sambrook and Russell, 2001).

2.2.14. Primer Dilution

Primers were supplied by macrogen company as a lyophilized product of different picomol concentrations and resuspending using deionized water to reach a final concentration for 100 picomols / µl of suspension, then diluted to10 picomols / µl according to the following equations (Chuang *et al*., 2013).

Dilution factor = ml, MW: Molecular Weight = µl

The amount of in µl of sterile deionized water was added to the tube of dried primer to obtain 100 picomol/ µl then diluted to 10 picomols/ µl by following the equation below.

Concentration 1 * Volume1= Concentration 2 * Volume 2

The primers were supplied by bioneer company as a lyophilized product of different picomoles concentrations, macrogen company protocol was adopted for primer resuspension, by bringing the final concentration of primers to 10 pmol/µl of deionized water.

2.2.14.1.Stoke Solution

Stock solution (100 pmol/µl) was prepared by adding nuclease free water or by adding ddH2O to the vial containing lyophilized primer while working stock of 10 pmol/µl was made by mixing 10 µl of the stock primer and 90 µl of ddH2O, and stored at-20 ºC.

2.2.14.2.Working Solution

Small aliquots were prepared at a suitable concentration and stored at -20 ºC until use to avoid repeated freezing and thawing.

2.2.15. PCR Amplification of Gene Sequences

Conventional polymerase chain reaction (PCR) was used to amplify a target DNA using specific primer pairs. Initially, PCR amplification conditions were optimized by separately varying, annealing temperature, primer concentration, and

DNA template concentration. Optimization of PCR reaction was accomplished after several trials as shown in table (2-9) for the first primer *lasI*/*lasR* 600/700 (Aghamollaei *et al.*, 2015), table (2-10) for the second primer *oprI*/*oprL* 249/504 (Jami Al-Ahmadi and Zahmatkesh Roodsari, 2016). Finally had been reached that the optimum temperature (annealing) for the first primer *lasI*/*R* 600/700 equal 57 °C and for the second primer *oprI*/*oprL* equal 57°C. Contents of the reaction in a mixture of PCR as shown table (2-11).

Table (2-9). PCR with primers *lasI* / *lasR* 600 / 700 bp

No.	Steps	Temperature	Time	PCR cycles
1	Pre-Denaturation	95°C	5 min	1
2	Denaturation	95°C	30sec.	30
3	Annealing	57°C-60°C-62°C	30sec.	
4	Extension	72°C	30sec.	
5	Final extension	72°C	5 min	1

Table (2-10) PCR with primers *oprI* / *oprL* 249/504 bp

No.	Steps	Temperature	Time	PCR cycles
1	Pre-Denaturation	95°C	5 min	1
2	Denaturation	95°C	1min	30
3	Annealing	57°C-60°C-62°C	1	
4	Extension	72°C	1	
5	Final extension	72°C	10 min	1

Table (2-11) Contents of the Reaction Mixture

NO.	Contents of reaction mixture	Component
1	Deionized water (Zero)	8.5 µl
2	Master Mix	12.5 µl
3	Forward Primer (10 pmole)	1 µl
4	Reverse Primer (10 pmole)	1 µl
5	DNA Sample (50 ng)	2 µl
6	Total Volume	25 µl

2.2.16. Detection of amplicons

Following amplification, aliquots (10 µl) were removed from each reaction and phage ladder 100 bp are examined by electrophoresis (70V and 60 min) in gels composed of 1.5% (w/v) agarose (Promega, USA) in 1X TBE buffer (40 mM Tris, 20 mM boric acid, 1 mM EDTA, pH 8.3), stained with ethidium bromide (2µl).

Gels were visualized under UV illumination using a gel image analysis system (Wu *et al*., 2004).

2.2.16.1.Agarose Gel Electrophoresis

PCR amplified products and a DNA molecular–weight marker (100bp DNA ladder) were electrophoresed on agarose gel to verify the size of the amplicon. PCR products were detected by agarose gel electrophoreses using Tris borate EDTA (TBE) as the running buffer. Agarose was weighted to prepare 1.5 % gel in 1X TBE in a glass conical flask. It was then placed in a microwave oven to melt the agarose completely, upon cooling approximately 4µl of 10 mg/ml ethidium bromide solution was added, and the molten agarose was poured into a gel casting tray with a gel comb. The comb was carefully removed. The running tray with gel was placed in the electrophoresis tank, and the gel was immersed in 1X TBE. The DNA molecular weight marker was also loaded in one of the wells (Lee *et al*., 2012). The power was set in the power supply usually at 60 voltages to resolve DNA under an electric field. Following electrophoresis, the products were visualized using UV light on a transilluminator and photographed.

2.2.16.2. Sequencing

The DNA samples of the genes with the primers were sent to acompany Macrogen company in the korea to perform the Sequencing.

2.2.17. Biosafety and Hazard Material Disposing

Biosafety aspects were followed during the work which includes disposing of all swabs, petridishes, and all contaminated supplies by autoclaving and then incineration. All benches cleaned with alcohol (70%) before and after the work. Wear a lab robe, gloves and a muzzle. Ium to *P. aeruginosa* (Bergen and Shelhamer, 1996).

2.2.18. Determine Levels of Cytokines

These tests were intended for quantification of serum levels of Interlukine -8 through the immuno enzymatic technique Enzyme-linked Immunosorbant Assay (ELISA) using Elisa washer and reader (Biotck/United State).

2.2.18.1.Determine Levels of Human Interlukin-8 ELIZA Kit

2.2.18.1.1. Test principle

This ELISA kit uses the Sandwich-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to Human IL-8. Samples (or Standards) are added to the micro-ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human IL-8 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human IL-8, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is proportional to the concentration of Human IL-8. You can calculate the concentration of Human IL-8 in the samples by comparing the OD of the samples to the standard curve.

The human IL-8 concentration of the sample can be interpolated from the standard curve, according to the manufactures company Elabscience / U.S.A.

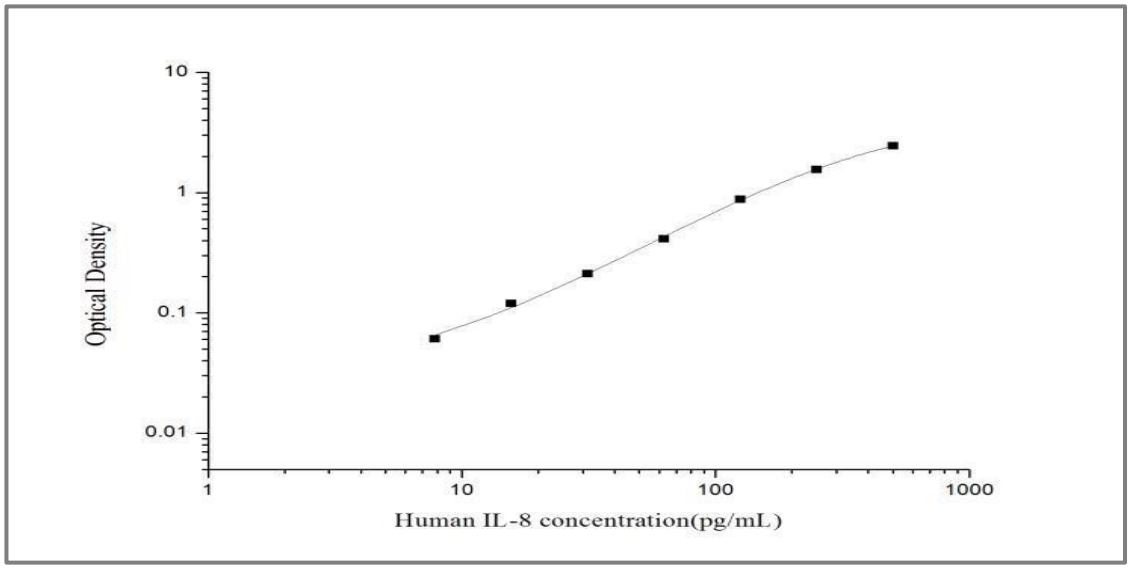


Figure (2-2). Standard curve of IL-8 concentration

2.2.18.1.2. Reagent preparation

All reagents were prepared according to the manufactures company Elabscience / U.S.A.

1- All reagents were placed at room temperature (18 - 25℃) before use.

2- Wash Buffer: 30mL was dilute of concentrated wash buffer with 720mL of deionized or distilled water to prepare 750mL of wash buffer.

3- Standard working solution: The reference standard solution was placed in a standard centrifuge at 10,000 × g for 1 minute. Then 1.0 mL of the reference standard and sample diluent were added, left standing for 10 minutes and then gently inverted several times. After it is completely dissolved, then mix well using a pipette. This reconstitution process results in a working solution of 500 gal/ml (or added 1.0 ml of the reference standard and sample diluent, let stand for 1-2 min then mix well with a low speed vortex meter, and bubbles generated during the vortex can be removed by centrifugation at a relatively low speed), serial dilutions were made as follows (500, 250, 125, 62.5, 31.25, 15.63, 7.81 0 gal/ml). Dilution method: 7 EP tubes were taken, and 500 µl of the reference standard and sample diluent were added to each tube. Then 500 µl of the 500 gal/ml working solution was added to the first tube and mixed to produce a 250

gal/ml pipette working solution 500 µl of solution from the previous tube to the last tube. and should be the last tube is empty. Do not suck the solution from the previous tube in it. the Figure (2-3). shows the method of dilution and prepare the standard solution.

4- Biotinylated Detection Ab working solution: The required amount was calculated before the experiment (100 µl/well). In preparation, was prepared slightly more than is calculated. Concentrated biotinylated detection Ab were centrifuged at 800 × g for 1 min, then, 100 × concentrated Biotinylated detection Ab was diluted to 1 × working solution with biotinylated detection Ab diluted (concentrated Biotinylated detection Ab: Diluted biotinylated detection Ab diluted = 1:99).

5- Horseradish peroxidase (HRP) Conjugate working solution: The required amount was calculated before the experiment (100µL/well). In preparation, was prepared slightly more than is calculated. Then, centrifuged the concentrated HRP conjugate were at 800×g for 1 min, then the 100× concentrated HRP conjugate dilute to 1×work in gsolution with HRP conjugate diluent (Concentrated HRP conjugate: HRP conjugate diluent = 1: 99).

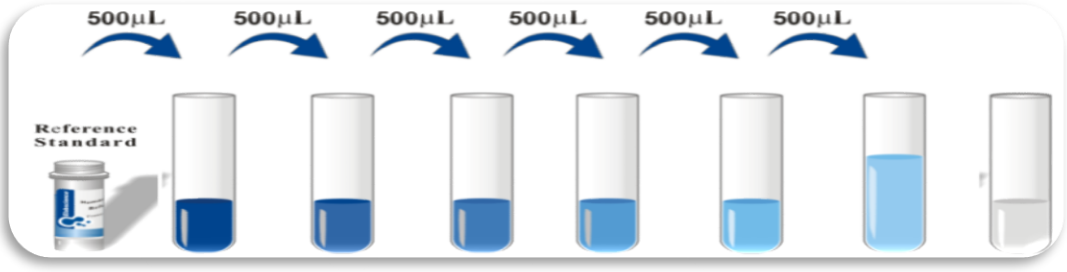


Figure (2-3). The method of dilution and prepare the standard solution that used in this study.

2.2.18.1.3. Assay procedure

1- Dilute, blank, and sample standard wells were determine.100 µl were added to each dilution of the standard, blank, and sample into the appropriate wells. The board was covered with the sealer, then it was incubated for 90 minutes at 37°C

2. liquid was decanted from of each well, and immediately 100 µl of Biotinylated Detection Ab working solution was added to each well. Then the plate covered with a new sealant. and incubated for 1 hour at 37°C.

3-The solution was decanted from each well, then 350 µl of wash buffer was added to each well. Soak for 1-2 min and then aspirate or decant the solution from each well and leave to dry on clean absorbent paper. The washing steps repeat this washing step. Then, the tested strips were made in use immediately after the washing step.

4- A 100µL of HRP Conjugate working solution was added to each well. Covered the plate with a new sealer. then it was Incubated for 30 min at 37°C.

5- The solution was decanted from each well, then the washing process was repeated 5 times as conducted in step 3.

6- A 90µL of Substrate Reagent was added to each well. plate was Covered with a new sealer. then it was Incubated for about 15 min at 37°C. It is essential here to protect the plate from light. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes then, the Micro plate reader was heated for about15 min before the OD was measured.

7- A 50µL of Stop Solution was added to each well.

8- Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

2.2.19. Statistical analysis

Statistical analysis was performed by SPSS computing program for the analysis of the results, $p<0.05$ was used as lowest significant limit and test (χ^2) was used. Statistical analysis was performed by using SPSS computing program version 23 for the analysis of the results (Kirkpatrick and Brooke, 2015).

Chapter Three

Results and Discussion

3. Results and Discussion

3.1. Data distribution

A total of 100 samples were collected from three different hospitals (Imam Al-Sadiq Hospital (peace be upon him) / Babylon, Al-Hussein Teaching Hospital / Karbala and Al-Sadr Medical City / Najaf). The study included 77 samples from Imam Al-Sadiq Hospital, 11 samples from Al-Hussein Teaching Hospital and 12 samples from Al-Sadr Medical City (figure 3-1).

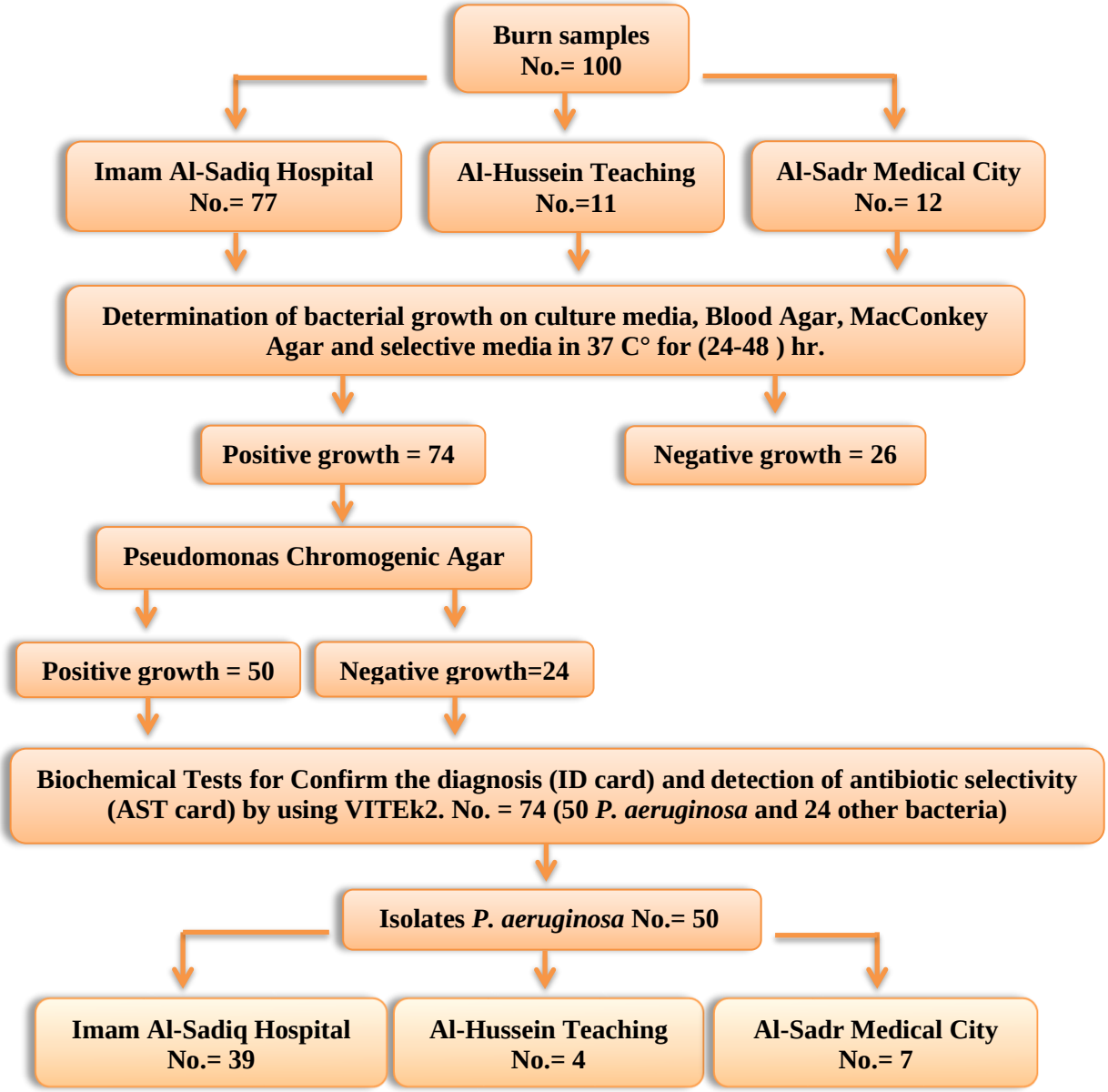


Figure (3-1). Isolation and identification of *Pseudomonas aeruginosa* isolates.

3.1.1. Distribution of burn patients according to the age

All samples were taken from burned patients with age from 1- 80 years for both sexes male and female (table 3-1).

Table (3-1). The distribution of burn patients according to the age.

Age group (years)	No. of patient (%)	χ^2	p-value
1-10	42 (42%)	**99.500**	**0.0001****
11-20	27 (27%)		
21-30	15 (15%)		
31-40	11 (11%)		
41-50	3 (3%)		
51-60	1 (1%)		
Above 61	1(1%)		
Total	**100**		

Table (3-1) showed the distribution of burn patients according to the age the most prevalent age group (1-10 years), followed by (11-20 years), (21-30 years), (31-40 years), (41-50 years), (51-60 years) and the least prevalence age group is those over (61 years) it represented by the following percentages (42%, 27%, 15%, 11%, 3%, 1%, 1%), respectively. There was highly significant differences ($P \leq 0.0001$) the increase cases in child (1-10 years) might be linked to several reasons like, lots of movement of children, a lack of awareness, and lack of knowledge of the nature of fire- causing materials and how to deal.

This result is in agreement with other study conducted by Agbenorku *et al*. (2011), which reported that scalds were seen commonly in children. And with the study from Palestine published by Tayh (2013) which showed that burn injuries in children (72%) were much more than burn injuries in adult (28%). Also, this study was consistent with Peck (2011) which found that the highest rate of burn injuries was in the children's group in the age group between (0 - 9) years, at a rate of 58 (54.2%) where deaths caused by burns occur among children at a rate of ten times in developing countries about it in the developed world.

Burns are one of the top fifteen major causes of child deaths, and the reason may be due to the neglect that children are exposed to while they are inside their parents' homes. Slovis (2011) found that the highest incidence of scald burn occurs among children under the age of five. Tekin *et al.* (2013) showed that 65% of cases have scalding burn and high rate of scalding burns was observed in the 0-5 year age group. Elsous *et al*. (2015) revealed that self-effect burns injuries carry significant morbidity and mortality between then appropriate younger persons.

3.1.2. Distribution of burn patients according to the sources, gender and hospitalization

Table (3-2). The distribution of burn patients according to the sources, gender and hospitalization.

Gender		Hospitals			Total	
		Imam Al-Sadiq	Al-Hussein Teaching	Al-Sadr Medical City		
Male	Children 1-12 year	25 (78%)	2 (6%)	5 (16%)	32(32%)	46 (46%)
	Adult 13-35 year	14 (100%)	0	0	14(14%)	
Female	Children 1-12 year	11 (79%)	3 (21%)	0	14(14%)	54 (54%)
	Adult 13-80 year	27 (67.5%)	6 (15%)	7 (17.5%)	40(40%)	
Total		**77 (77%)**	**11 (11%)**	**12 (12%)**	**100**	

Table (3-2) showed the distribution of burn patients according to the hospitals, the highest percentage of burn patients was (77%) in the Imam Al-Sadeq (peace be upon him) hospital compared to Al-Hussein Teaching Hospital (11%) and Al-Sadr Medical City (12%). Burn injuries according to gender, most commonly observed in females 54(54%) in comparison to that of males which 46(46%). This was consistent with the results of Farhood and Chelab (2017) which found that the number of diagnosed cases of burn injuries in female were 61 cases, with a percentage of 57% while there was 46 cases of burns for males with a percentage of 42.9%. Church *et al.* (2006) found that the percentage of isolates of 63% for females and 36% for males. This is related with nature of women's work at home

and their preoccupation with household chores, especially with regard to cooking or near sources of fire, liquids and hot fumes. Also, in other studies from developing countries such as Zambia, South Africa, Malawi, Peru, Turkey and many countries (Mukerji *et al.*, 2001; Peck *et al.*, 2008; Agbenorku *et al*., 2011; Aliosmanoğlu, 2011; Samuel *et al.*, 2011). Big majority of females were housewives and scalding burns were frequently encountered in females. It also agrees with the study conducted by Panjeshahin *et al.* (2001) in Iran in which females were the victims of burns more frequently than males. They attributed the high number in females to the following reasons: First, most of females were housewives with low level of literacy, as these people mainly work at kitchen. Second, traditionally the style of females' clothes which has a higher volume compared to European females' clothes. Third, the material of females' clothes is mostly synthetic type comparing to the males' clothes suggesting that the females' clothes are more easily flammable.

This is in contradiction to a study conducted in Morocco by Essayagh *et al.* (2019) which reported a higher incidence of burn injuries in male (64%) than in female (36%). Also, Gayathri *et al.* (2015) found that the incidence of males is greater than that of females, as the percentage of males was 54%, while the percentage of females was 46%. Sharmeen *et al.* (2012) found that largest death from burns in new married women in India and most middle East its consider a big problems. The reason is back open fire on bread and cooking by ovens called tandoor were traditional habits of women were living in rural areas and some areas of city. On the other hand It can be concluded that children, female homemakers, and workers in the Iraq society are at a higher risk of burns.

3.1.3. Gender distribution of burned patients according to the cause of burn

Table (3-3). Gender distribution of burned patients according to the cause of burn.

Gender		Cause of burn (%)			Total cases (%)		χ^2	p-value
		Fire	Liquid	Electricity				
Male	Children	12(37.5%)	19(59.4%)	1 (3.1%)	32 (32%)	46 (46%)	12.789	0.04*
	Adult	5(36%)	8(57%)	1 (7%)	14 (14%)			
Female	Children	0	14(100%)	0	14 (14%)	54 (54%)		
	Adult	24(60%)	16(40%)	0	40 (40%)			
Total		41(41%)	57(57%)	2 (2%)	100			
p-value	0.002*							

* Significant

Table (3-3) showed the gender distribution of burned patients according to the cause of burn. The results of the current study showed that the most common cause of burns in burn patients was liquid material burns 57(57%) and included (hot water, milk, tea, petrol, oil, and gasoline), followed by fire burns 41(41%) and electricity burns 2(2%).

The results showed that the percentages of liquids burns in men [children 59.4%, adult 57%] and the percentages of liquids burns in women [children 100%, adult 40%] and this shows that women are more exposed to liquid burns (scalding) than men, followed by fire burns (flame), where the percentages in men were [children 37.5%, adult 36%] and in women [children 0%, adult 60%] his is also prevalent in women compared to men, followed by electrical burns in men [children 3.1%, adult 7%] and in women 0% which showed that they are more prevalent in men than in women. ($p \leq 0.05$). The results of the current study are almost in agreement with a study conducted in Palestine by Tayh (2013) which showed that the accidents of hot liquids (scalds) (66.1%), followed by fire (33.9%), this may be explained based on the fact that hot liquids are of high importance at our homes (where women and children usually exist) and most frequently used in many life aspects. Farhood and Chelab (2017) found that the highest rate of burn injury was burning with boiling water by 38.3%, followed by burning by gas flame with a rate of 28.9%, and the lowest percentage of burn

injury was electrical burns by 4.67%. Yousefi-Mashouf and Hashemi (2006) mentioned that the highest burning rate was with boiling water by 23.4%, then burning by gas flames by 14.5%, and the lowest burning rate was by electric burns by 4%.

The current study was consistent with the results of two Iranian studies that reporting the most prevalent causes of burns in men as oil and its products, in children and in women as scalds (Ahmadi *et al.*, 2006; Goodarzi *et al.*, 2014). Essayagh *et al.* (2019) reported that the predominant burn agent to be gas flame, followed by scalding liquid and contact with an electrical source. The results of the current study did not agree with study of American Burn Association (2019) reported that, overall, flame burns are still the majority of injuries in the USA (41%), with scalds second at (31%), Chemical (3.5%) and electrical burn injuries (3.6%) occur much less commonly. Samuel *et al.* (2011) Which stated that the highest rate of injury was by burning by gas flames, followed by burning by boiling water burns rate was 29%, followed by electric burns by 18%. Also, did not agree with the study conducted by him Irfan *et al.* (2014) in which it was stated that the highest incidence of gas burns was 29%, followed by electrical burns by 18%.

According to gender, the present study revealed that burns are more prevalent in female homemakers workers and children, were more likely to get burns due to hot liquids (scalds). Samimi *et al.* (2011) reported that most the burns to be more in children, and Stampolidis *et al.* (2012) reported burns to be more prevalent in female homemakers and workers. With regard to the findings of the present study and other studies, it can be concluded that although the causes of burns are known and several, the people are not cautious and ignore the safety instructions and standards in the use of flammable and explosive materials.

3.2. Bacterial growth and percentage in burns

The prevalence of bacterial growth in collected swab sample was 74% while growth negative was 26%, (figure 3-2).

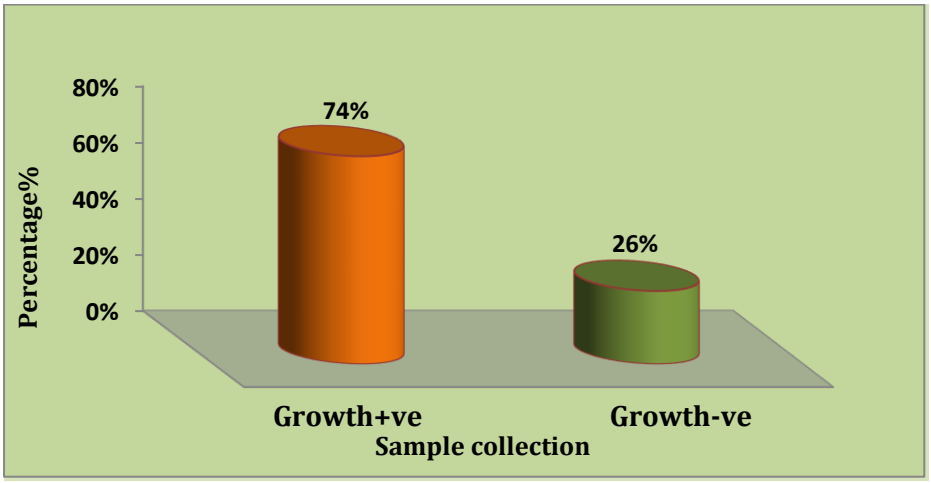


Figure (3-2). Distribution of growth positive bacteria in collected samples.

Severe burns are one of the serious forms of trauma, including loss of the skin barrier and tissue destruction. Indeed, tissue injury at burn sites results in the production of biological fluids which defined as burn wound exudates (BWEs) (Oncul *et al.*, 2009; Gonzalez *et al.*, 2016). The immunosuppression state and burn tissue microenvironment are favorable features for burn wound pathogens colonization and proliferation which lead to the spread and growth of different type of bacteria in the burn area according to the degree of burning of the body (Gonzalez *et al.*, 2016).

Table (3-4) Explained the relationship between cultures results for male and female burn patients and the degree of burn. The results of the current study showed that the highest percentage of burns degrees was in patients with second-degree burns 54%, followed by third-degree burns with a percentage 45%, and finally first-degree burns with a percentage of 1% in both males and females. According to positive culture the third-degree burns were more positive with a percentage of [male 42.2% , female 44.4%] comparing to the positive cultures of second degree burns with a percentage of [male 26%, female 37%] and the

positive cultures of first degree burns with a percentage of [male (0) , female 100%].Therefore the relationship between the degree of burn and gender showed no significant differences.

Table (3-4). Distribution of burn degree among studied patients according to the gender.

Burn of degree	Negative		Positive		Total	χ^2	p-value
	male	female	male	female			
First degree	0	0	0	1(100%)	1(1%)	**0.205**	**0.903**
Second degree	10(18.5%)	10(18.5%)	14(26%)	20(37%)	54(54%)		
Third degree	3(6.7%)	3(6.7%)	19(42.2%)	20(44.4%)	45(45%)		
Total	**13(13%)**	**13(13%)**	**33(33%)**	**41(41%)**	**100**		
	26(26%)		**74(74%)**				

Tayh (2013) showed that the third-degree burns were more positive 61.5% compared to positive second-degree burns 42.4%, (the high burn percent size and degree increase the chance of pathogenic organisms' colonization). As the same time, the chance of occurrence septicemia increases with increasing burn degree patients with burn of third degree are more prone to septicemia and (mortality) than patients with burn of second degree. This indicates that an occurrence of septicemia and mortality are influenced by burn degree Hodle *et al*. (2006) detected that the depth of burn considered as risk factor for the occurrence of septicemia in burned patients.

Patient with full-thickness burns is more difficult and takes long time even return to normal state, as the increase in the time of hospitalization leads to the risk of nonsocial infection, ultimately increasing rate of mortality and morbidity (Aldemir *et al.*, 2005). Patients who had deeper and more extensive lesions lead to more colonization of burn wound and then to septic and death (De Macedo *et al*., 2003). Other studies demonstrated that a significant association between increasing burn size and increasing incidence of pathogenic organisms (Komolafe *et al.*, 2003; Oncul *et al.*, 2009). They also reported that the incidence of invasive-cultures increased as burn size increased.

3.3. Bacterial culture types

The growth and types of bacteria vary over time in the burn area from one person to another, where when victims arrive at the hospital after a criminal or accidental accident, An initial culture was done to diagnose whether they had a bacterial infection or not. This is related to the patient's delay in arriving at the hospital or not coming directly. (Most of the culture results confirmed that most of the patients from whom samples were collected showed bacterial infection after the third day of admission).

This supports the findings of Al-Musawi and Al-Garawi (2015) who found that 66% of skin swabs gave positive growth culture for three days in the hospital, while the result was 88% after seven days of hospitalization. On the other hand, the results of the current study proved that there was a positive relationship between a longer stay in hospital and the high prevalence of pathogenic bacteria causing burn infections. Contaminated burning wards and duration of patients stay in hospital, in addition to the size of surface area of burned skin are the most important reasons to increase of persistent and multiplication of pathogenic bacteria in the burned areas (Al-Aali, 2016).

Table (3-5). Gram negative bacterial isolates obtained from burned patients in adult and children.

Isolated bacteria	Male N(%)		Female N(%)		Total Number
	Adult	Children	Adult	Children	
Pseudomonas aeruginosa	4(8%)	19(38%)	21(42%)	6(12%)	50(67.6%)
Klebsiella	1(11.1%)	2(22.2%)	5(55.6%)	1(11.1%)	9(12.2%)
E. coli	2(25%)	2(25%)	2(25%)	2(25%)	8(10.8%)
Proteus	1(33.3%)	0	2(66.7%)	0	3(4%)
Acinetobacter	0	1(50%)	1(50%)	0	2(2.7%)
Citrobacter	1(50%)	0	1(50%)	0	2(2.7%)
Total summation	**33(45%)**		**41(55%)**		**74(100%)**
χ^2	**22.561**				
p-value	**0.026***				

Table (3-5) showed the gram-negative bacterial Isolates obtained from burned patients. The results showed the numbers and percentages that were obtained from

clinical samples of burn patients of both sexes, males and females, which showed high levels of *p. aeruginosa* bacteria, followed by *Klebsiella*, *E. coli*, *Proteus*, *Acinetobacter* and *Citrobacter* they represent the following percentages 50 (67.6%), 9(12.2%), 8(10.8%), 3(4%), 2(2.7%) and 2(2.7%).

According to gender the results obtained showed that the prevalence of *P. aeruginosa* bacterial isolates was more in women [adult 21(42%) , children 6(12%)] than in men [adult 4(8%) ,children 19(38%)] ($p \leq 0.05$) This result agrees with the other result obtained by Singh *et al.* (2017), they found that the *P. aeruginosa* is the most common source of burn wound infection. And also agree with Nikokar *et al.* (2013) they mentioned that the high frequency rate of *P. aeruginosa* found in burn units might be due to the prolonged hospital stay and intensive use of antibiotics. The studies of Kirketerp-Møller *et al*. (2011) and Mhada *et al.* (2012), revealed the predominant organisms isolated from burns wounds were *Pseudomonas aeruginosa* [35.84%], *Klebsiella* species [27.30%], *Acinetobacter* species [20.13%], *Escherichia coli* [2.38%], *Staphylococcus aurous* [8.87%]. Farhood and Chelab (2017) showed that the highest isolate rate was for *P. aeruginosa* bacteria with an isolate rate of 38 (32.47%), followed by *K. pneumonia* bacteria with an isolated percentage of 25(21.36) and the lowest percentage of infection with *S.epidermis* bacteria With an isolate rate of 2(1.7), while the percentages of the following bacterial species *A.baumenii*, *E.coli*, *E.cloacae*, *S.auraus*, *B.cepesa*, *P.miribilles*, *P.agglomer* were 15 (12.82%), 13(11.1%), 6(%5.12), 6(5.12%), 5(4.27%), 3(2.56%) respectively. These bacteria are considered opportunistic pathogens and rarely cause disease in healthy people, but they are highly virulence in patients with weak defensive mechanisms causes bacteremia, and therefore the contamination in hospitals with these pathogens have a pathological effect to deteriorate the condition of those sleeping there (Brown *et al.*, 2012). Boyer *et al.* (2011) found that the isolates of *P. aeruginosa* bacteria were with an rate of 43 (41.3%). Also, nearly similar to the study of Kanagapriya *et al.* (2015) where he found the percentage of isolates of *P. aeroginosa*,

K.pneumonia, E.coli, and *P.miribila* bacteria was 28%,20%,8%,4%, respectively. Tayh *et al.* (2016) mentioned that the percentage of isolates of *P. aeruginosa*, *K.pneumonia*, *E.claocae,* and *A.baumannii* bacteria were 37.50%, 25%, 10%, and 5%, respectively.

Whereas Jithendra *et al.* (2015) that found, an increase in the rate of isolation of *S.aureus* bacteria in the first place, with an isolation rate of 39.8%, then followed by *P. aeruginosa* bacteria with an isolation rate of 35.3%. The reason may be due to the number of samples Which were included in the study or according to the geographical location, it varies from one location to another and from one hospital to another, as these bacteria were not found in this percentage in another hospital in the same city. George *et al*. (2015) found that the isolation percentage of *S.aureus, P.aeroginosa*, *K.pneumonia*, *E.coli, A.baumenii* was 39.4%, 14.2, 13.4, 8.7, 7.9 respectively.

According to gender, the results of the current study confirmed the prevalence of *Pseudomonas aeruginosa* bacteria in women more than men. And this result is agree with a study conducted in Iraq, Karbala city by Alkateeb *et al.* (2016), and with the results of Kireçci and Kareem (2014) in the city of Sulaymaniyah, Iraq and Shewatatek *et al.* (2014) in Ethiopia. The results indicated a higher incidence of the bacterium in female and elderly patients.

Whereas, Chand *et al.* (2020) found the prevalence of *P. aeruginosa* isolates was 4.29%, in which the distribution in male patients 56 (64.36%) was higher than in female patients 31 (35.63%). It also contradicts Mokhtari and Amini (2019) which indicated that the percentage of *P. aeruginosa* in males was (53%) and in female patients (47%) and the highest percentage (28%) ranged between 24-29 years compared with the elderly, difference between the results of the current study and the results of previous studies is due to the possible reasons types of studied populations, different geographical locations, type of hospitals (Manandhar *et al*., 2018).

The current study showed a clear predominance of *P. aeruginosa* bacteria, and the prevalence of this bacteria may be due to its resistance to antibiotics and antiseptics, and the transformation of the burn area into a suitable medium for the growth of these bacteria due to the weak resistance of the skin tissues subject to burning and damage, in to the presence of this bacteria in abundance in the environment surrounding the patient in the burn unit or the nursing staff In hospitals, in to their presence in abundance on the number and medical supplies, in to the severity of overcrowding in the burn unit at times (Mooij *et al.*, 2007). It was observed when comparing the current results with the local and global results that there is a convergence and difference in the rates of isolation from different samples, and this is due to many reasons, including the variation in the number of samples collected by the researcher, as well as the degree of cleanliness and the type of sterilizers and disinfectants used in hospitals, as well as the difference in hygiene habits in each country. The study showed that *P. aeruginosa* was the most common bacterium from burned isolates and that its resistance to antibiotics was high, which requires careful monitoring of these microbes through continuous programs and activation of infection control committees in hospitals and the need to rethink the way to deal with infections according to health regulations applicable.

3.3.1. Isolation and identification of *pseudomonas aeruginosa*

The diagnosis was made based on the phenotypic characteristics of the bacterial isolates on each of the culture media that were used in the diagnosis, which is represented by the medium of MaCconkey agar, as the bacterial colonies were appeared as pale in color and not fermented the sugar lactose (lactose non-fermentation) (Forbes *et al*., 2002; Baron *et al* ., 2007). Either on blood agar medium, the β-hemolysis bacterial colonies appeared, evidence of the production of hemolysin enzyme. (Selim *et al.*, 2015; Procop *et al.*, 2020). And on Pseudomonas chromogenic agar medium which is a selective medium for *P.*

aeruginosa incubated at 37 ºC during 24-48 hours, the bacterial colonies were appeared as magenta in color and the color of the medium that change from green to blue-green (Figure 3-3). Many studies found that the chromogenic agar for *P. aeruginosa* is promising medium for direct isolation and identification with high sensitivity and specificity (Laine *et al.*, 2009; Momin *et al.*, 2017). In addition to, Pseudomonas chromogenic agar will not only aid routine to detect *P. aeruginosa* rapidly using only one media, but it will also provide the opportunity to conduct such procedures in a cost-effective and reliable manner (Sivri *et al.*, 2014). As well as Pseudomonas chromogenic agar is a promising medium allowing for the isolation and simultaneous identification of *P. aeruginosa* from in burn infection (Al-Dahmoshi *et al.*, 2018).

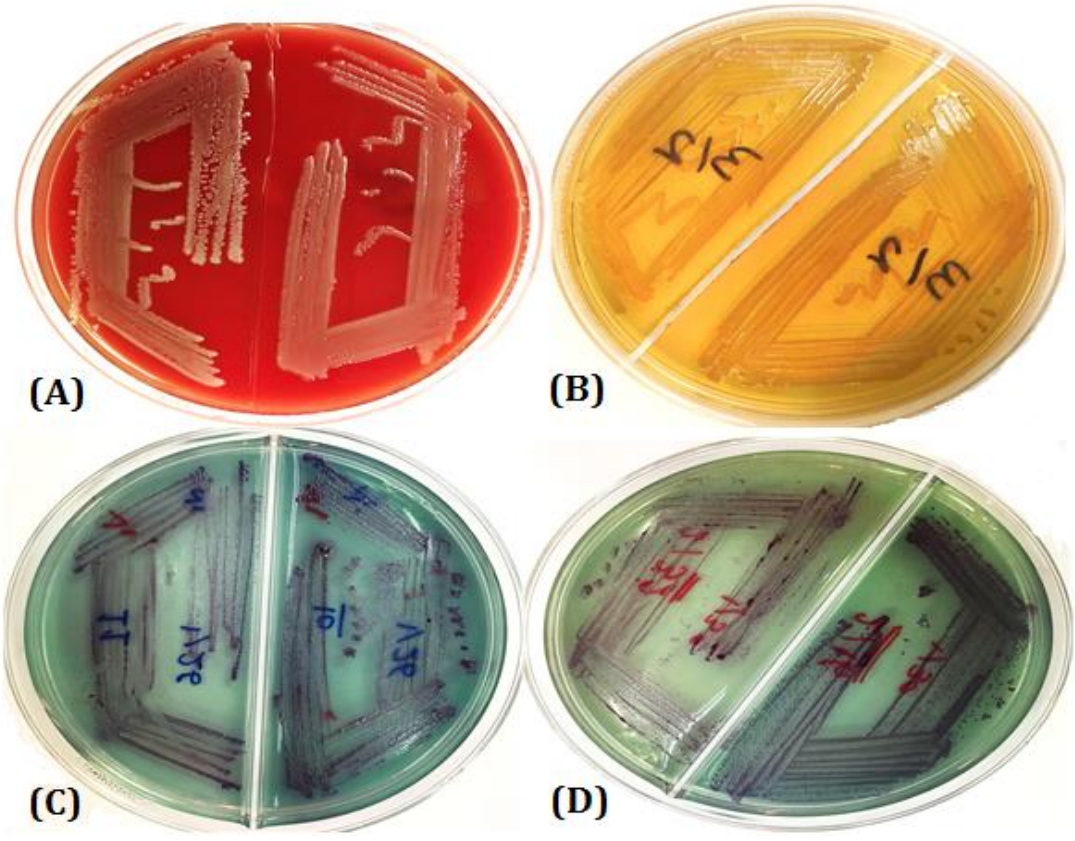


Figure (3-3). *pseudomonas aeruginosa* colonies on Culture Media. (A): *pseudomonas aeruginosa* isolate on Blood agar medium. (B): *pseudomonas aeruginosa* isolate on MacConkey agar medium. (C) and (D): *pseudomonas aeruginosa* isolates on Pseudomonas Chromogenic agar medium.

3.3.1.1. Conformational identification by VITEK2 GN ID card System

Diagnosis of *pseudomonas aeruginosa* isolates depends on the colonial morphology, biochemical tests, VITEK2 GN/ID card system and molecular identification. After the colonies of *P. aeruginosa* were grown on culture media (MacConkey agar / Pseudomonas Chromogenic agar) as shown in the figure (3-4 B,C,D), diagnosis confirmed by using VITEK2 system, It is one of the best systems and devices to identify bacterial species in a short period and very accurately and was developed by the French company Biomerieux, characterized by fast detection of bacteria without the need for many of culture media as well as reduce cultural contamination, through the using GN-ID cards which contained 64 biochemical tests. Table 3-6 demonstrate that fifty isolate of *pseudomonas aeruginosa* were confirmed with level excellent.

Table **(3-6). Identification results *pseudomonas aeruginosa* by VITEK2 GN-ID card system**

Selected Organism			99% Probability		**Pseudomonas aeruginosa**												
			Bionumber: 0003053103500352										**Confidence:**		Low discrimination		
SRF Organism																	
Analysis Organisms and Tests to Separate:																	
Pseudomonas aeruginosa	42C(99),PYOCYANIN(99),																
Pseudomonas putida	42C(1),PYOCYANIN(1),																
Low Discrimination Organism																	
Analysis Messages:																	
Contraindicating Typical Biopattern(s)																	
Pseudomonas putida	BAlap(21),																
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	+			

3.4. Antibiotic Susceptibility testing (AST)

The sensitivity of *Pseudomonas aeruginosa* isolated from burn infections to antibiotics was tested by using the VITEK2 AST- GN76 card system for Gram-negative bacteria which contains 17 antibiotics, through it, the bacteria are characterized and the antibiotics that affect them are identified within 5-8 hours,

thus reducing the manpower needed to complete the research task. Completely, there is no human involvement in it, and it gives results in a way that makes it easy for the researcher to use and build on them. The accuracy of the diagnosis for the VITEK2 system is about 99%. MDR of *P. aeruginosa* isolates were represented by resistance to a number of antibiotics (Table 3-7, Figure 3-4 and Appendix1).

Table (3-7). The results of antibiotic susceptibility tests by VITEK-2 system for *Pseudomonas aeruginosa* isolated from burn patients

Target action of Antibiotics in clinical Isolates	Family	Antibiotic	Symbol	Zone diameter(mm) (No. = 50 pseudomonas isolate)			Total
				S (%)	I (%)	R (%)	
Cell wall Synthesis	Betalactamase	Piperacillin/ Tazobactem	TZP	50 (100%)	-	-	50
	Carbapenems	Imipenem	IPM	6 (12%)	-	44 (88%)	
	Cephalosporins (I,II,III,IV)	Cefepime	FEP	6 (12%)	7 (14%)	37 (74%)	
		Cefazolin	CZ	-	-	50 (100%)	
		Ceftazidime	CAZ	15 (30%)	7 (14%)	28 (56%)	
Protein Synthesis	Tetracyclins	Tigecycline	TGC	2 (4%)	-	48 (96%)	
	Aminoglycosides	Amikacin	AN	6 (12%)	1 (2%)	43 (86%)	
		Gentamicin	GM	5 (10%)	3 (6%)	42 (84^)	
Nucleic Acid Synthesis	Fluoroquinolones	Levofloxacin	LEV	7 (14%)	-	43 (86%)	
		Ciprofloxacin	CIP	3 (6%)	45 (90%)	2 (4%)	

Table (3-7) showed that the results of antibiotic susceptibility for *P. aeruginosa* isolated from burn patients where the results showed that the highest resistance of *P. aeruginosa* to Cefazolin with a percentage 100% followed by Tigecycline 96%, Imipenem 88%, Amikacin and Levofloxacin 86%, Gentamicin 84%, Cefepime 74%, Ceftazidime 56% and Ciprofloxacin 4%. Whereas some isolates of *P. aeruginosa* observed intermediate resistance 90% to Ciprofloxacin. On the other hand, the results of some *P. aeruginosa* isolates showed a high sensitivity to Piperacillin-Tazobactem with a percentage 100%.

The results showed that the resistance of *P. aeruginosa* to Cephalosporins represented by cefazolin 100%, cefepime 74% and ceftazidime 56%, this result was close to that of Javiya *et al.* (2008) who reported a resistant rate of *P. aeruginosa* cefazolin (83.93%), cefepime (69.64%). and close to other results

obtained by Shahandashti *et al.* (2012) who recorded cefazolin (92.3%). Also, close to the result obtained by Bhatt *et al.* (2015) where the percentage of resistance to cefepime (64.6%). This result was very close to the results of Ranjbar *et al.* (2011) who reported a resistance rate of *P. aeruginosa* Ceftazidime (57.5%). And the results obtained by Kianpour *et al.*(2010) and Hussein *et al.* (2018) who recorded resistance to rates Ceftazidime (53.57%, 55.5%,), respectively.

The results of the current study also showed a high sensitivity of the combination of beta-lactamase inhibitors represented by Piperacillin/ Tazobactem 100%. When the beta-lactam antagonist piperacallin is combined with the beta-lactamase inhibitor tazobactam, the beta-lactamases initiate an intrinsic resistance mechanism that leads to the inactivation of the Beta-lactams, making them inactive. Beta-lactamase inhibitor such as tazobactam (An irreversible inhibitor of a wide variety of bacterial beta-lactamases) can improve many beta-lactamases such as piperacillin once combined with them. Piperacillin-tazobactam is the β-lactamase inhibitor combination most commonly used to treat *P. aeruginosa* infection (Tannous *et al.*, 2020; Al Muqati *et al.*, 2021).

Beta-lactam Beta lactamase inhibitor combination, piperacillin-tazobactam which revealed high susceptibility to *P. aeruginosa* so, thought that the result due to many mechanisms; First: Piperacillin one of β-Lactams antibiotic bear structural resemblance to a natural substrate of penicillin-binding proteins (PBPs), i.e., the dipeptide D-alanyl-D-alanine, allowing them to effectively bind these enzymes. At the PBP active site, a serine residue attacks the carbonyl carbon of the β-lactam, resulting in the formation of a covalent acyl-enzyme complex that is slowly hydrolyzed (Zapun *et al* .,2008). PBP inhibition impairs peptidoglycan cross-linking, thereby leading to deregulation of bacterial cell wall synthesis and activation of cell lysis (Sauvage *et al.*, 2008 ; Goo and Sim, 2011). The determination of PBP inhibition profiles is therefore important for establishing β-lactam activity against a given species. In the case of *P. aeruginosa*, the targets of

β-lactams are the PBPs essential for cell viability, namely PBP1b, PBP1c, PBP2, and PBP3. Also, noteworthy is the non-essential PBP, PBP4, whose inhibition triggers a highly efficient and complex β-lactam resistance response and hence serves as a trap target for β-lactams (Moya *et al*., 2009 ; Moyá *et al*., 2010).

These resistance mechanisms have resulted in strains resistant to available antipseudomonal agents, including β-lactams, fluoroquinolones and aminoglycosides, and have greatly compromised the clinical efficacy of these agents (Juan *et al*., 2010 ; Perletti *et al*., 2010). Second: Tazobactam is a sulfone derivative of penicillanic acid (Beale and Block, 2011) Like other early β-lactamase inhibitors (e.g., clavulanic acid, sulbactam), the moiety at position 1 (a sulfone group in tazobactam) acts as a leaving group that promotes secondary ring opening at the β-lactamase active site, thereby facilitating covalent bond formation between tazobactam and the enzyme, and subsequently leading to irreversible inhibition (Drawz and Bonomo, 2010). Tazobactam is an inhibitor of most class A β-lactamases (including many ESBLs (extended-spectrum β-lactamases) and some class C β-lactamases (cephalosporinases) under the Ambler classification scheme (Drawz and Bonomo, 2010; Beale and Block, 2011). At the β-lactamase active site, tazobactam forms a stable imine acyl-enzyme complex that undergoes hydrolysis much more slowly than the complex formed by β-lactams to eventually free the enzyme (transient inhibition) (Beale and Block, 2011).

Often referred to as an irreversible or “suicide” β-lactamase inhibitor, tazobactam actually undergoes multiple fates after the formation of this complex: (1) deacylation of the complex to regenerate the active enzyme and an inactive product; (2) tautomerization of the imine to form an enamine, also a reversibly inhibited enzyme; and (3) the formation of an irreversibly inactivated enzyme after a series of degradation reactions (Yang *et al*., 1999). The functional inhibition of the enzyme is determined by the relative rates of each of these pathways (Drawz and Bonomo, 2010). So extended-infusion piperacillin-tazobactam therapy is a

suitable alternative to intermittent-infusion piperacillin-tazobactam therapy, and they strongly suggest that improved outcomes may be realized by administering extended-infusion piperacillin-tazobactam therapy to critically ill patients with *P. aeruginosa* infection (Lodise *et al*., 2007). Also, The high susceptibility against antibiotics Piperacillin/ Tazobactem than other antibiotics, this result might be due to that such antibiotics recently used in clinical field, and have no long used against bacterial infections , as well as the mode of action of such antibiotic has ability to bind to LPS of G-ve bacteria and increased cell membrane permeability , and enhance antibiotic uptake Also, the results showed the resistance of *P. aeruginosa* to Carbapenems represented by the antagonist Imipenem 44(88%). Imipenem result was close to that of (Ranjbar *et al.*, 2011; Parsa *et al.*, 2020) who reported the percentage of resistance was (97.5%, 97.1%) respectively. And (Mardaneh *et al*., 2013; Kombade and Agrawal, 2014) who recorded resistance (85%, 86.9%) respectively. But on the other hand it differs from (Kianpour *et al*., 2010; Bhatta *et al.*, 2019) who found that the highest resistance was (14.28%, 10.5%), respectively.

Although among available antibiotics, Carbapenems (Meropenem, Ertapenem, Doripenem, Imipenem) are commonly used to treat *P. aeruginosa* infections, their widespread application has increased the resistance of this organism to these antibiotics. Nevertheless, some resistant strains to Carbapenem have been detected, which are defective in the expression of OprD (Rossolini and Mantengoli, 2005; Li *et al.*, 2012). In addition, the porin OprD is known to promote the internalization of Imipenem and to some extent, Meropenem but not of other β-lactams. Thus, the modification of OprD structure and/or the reduction of its expression confer reduced susceptibility to Imipenem. The alteration of OprD is often associated with overexpression of efflux systems, thus conferring a high level of resistance to Imipenem, but also to other classes of antibiotics such as quinolones and aminoglycosides (Bassetti *et al.*, 2018). Also, detected reasons for

resistance of *P. aeruginosa* isolates to carbapenem include: reduced the permeability of the outer membrane, increased expression of efflux pumps, changes in binding proteins and the presence of carbapenem hydrolyzing enzymes such as metallo beta lactamases (Mahmmudi *et al.*, 2016).

Aminoglycoside's resistance, such as Amikacin and Gentamycin. the resistance rate to Amikacin recorded in this study was 43(86%) and Gentamicin 42(84%). This result was consistent to the results Parsa *et al.* (2020) who reported Amikacin (95%) and Gentamicin (97.1%). And Ranjbar *et al.* (2011) which was found Amikacin (90%) and Gentamicin (67%). And Bhatt *et al.* (2015) which was found Amikacin (73.2%) and Gentamicin (84%). Also, Mokhtari and Amini (2019) which was found Amikacin (80.3%). On the other hand, the results of this study were far from Vitkauskienė *et al.* (2010) that was found Gentamicin (37%). And were far from Alramahy and Aladily (2017) that was found Amikacin (26%). Amikacin, and Gentamicin are aminoglycosides antibiotic. Acquired resistance to aminoglycosides is mediated by transferable aminoglycoside-modifying enzymes (AMEs), rRNA methylases and derepression of endogenous efflux systems. Modification and subsequent inactivation of aminoglycosides are achieved by three deferent mechanisms: (1) acetylation, by aminoglycoside acetyltransferases (AACs), (2) adenylation, by aminoglycoside nucleotidyl-transferases (ANTs), and (3) phosphorylation, by aminoglycoside phosphoryl transferases. Methylation of the 16S rRNA of the A site of the 30S ribosomal subunit interferes with aminoglycoside binding and consequently promotes high-level resistance to all aminoglycosides (APHs) (Meletis and Bagkeri, 2013).

The resistance of the fluoroquinolones in the current study showed 43(86%), 2(4%) to Levofloxacin and Ciprofloxacin, respectively, while intermediate resistant to Ciprofloxacin was 45 (90%). This result is nearly compatible with the data reported by Parsa *et al.* (2020) he record (96.2%) of isolated were resistance to Ciprofloxacin, but disagree with that reported by Kombade and Agrawal (2014)

he record (34.8%) resistance. Either for Levofloxacin (4%) This rate was far from the results of Yayan *et al.* (2015) (30.6%) and Lila *et al.* (2017) (36.1%). Fluoroquinolone antibiotics such as Ciprofloxacin and Levofloxacin interfere with DNA replication by inhibiting DNA gyrase and topoisomerase IV (Pang *et al.*, 2019). Ciprofloxacin and Levofloxacin resistance can arise through the acquisition of mutations in genes encoding the target proteins of Ciprofloxacin and regulators of efflux pumps, which leads to overexpression of these pumps leading to increases the expulsion of Ciprofloxacin from *P. aeruginosa* cells and occurs through mutations in regulatory genes of efflux pumps (Rehman *et al.*, 2019). The resistance of Tetracyclines represented by Tigecycline showed a high resistance of *P. aeruginosa* bacteria (96%). This result is close to Somily *et al.* (2012) where it was found that the proportion of Tigecycline (84.9%).

Pseudomonas aeruginosa showed resistance to a wide range of antibiotics, comprises aminoglycosides, quinolones and β-lactams. The resistance may be intrinsic (low outer membrane permeability, coding for efflux pumps and the making of antibiotic-inactivating enzymes), acquired (either horizontal transport of resistance genes or mutational alteration) and adaptive (involves formation of biofilm which provide as a diffusion barrier to edge antibiotic access to the bacterial cells) resistance (Mulcahy *et al.*, 2010; Breidenstein *et al.*, 2011). Also, the prevalence of resistance has often genetic origins and is due to add or remove the genetic fragments. This leads to changes in the pattern of chromosome sets in isolates of *P. aeruginosa*. As a result, precise examinations of drug-resistant infectious agents are required for proper treatment of bacterial infections and then prescribe medications. Understanding the state of sensitivity and resistance of these bacteria in hospitals is a fundamental measure in order to determine the therapeutic plan in early dealing and control the bacteria's resistance to antibiotics (Mahmmudi *et al.*, 2016) Also, the development of antibiotic resistant towards *P. aeruginosa* might be due random use of antibiotics, production of different types

of enzyme like carbapenamase, AmpC-lactamases, quorum Page 8/12 sensing modification of different target side etc. (Javiya *et al.*, 2008; Nikbin *et al.*, 2012). Furthermore, one of the major cause of the emergence of *P. aeruginosa* is prescribing the antibiotics without performing susceptibility test due to lack of laboratory facilities in most of the healthcare centers (Anil and Shahid, 2013 ; Shrestha *et al.* 2015). The figure (3-4) shows distribution of resistance *P. aeruginosa* isolates for antibiotics used in this study.

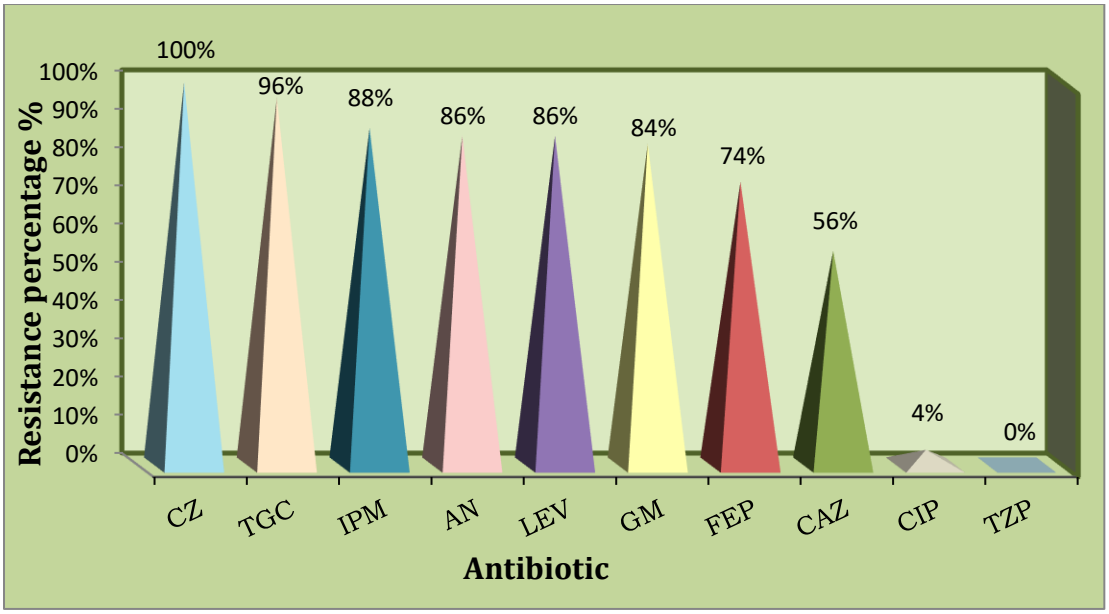


Figure (3-5). Distribution of resistance *P. aeruginosa* isolates for Antibiotics., CZ: Cefazolin, TGC: Tigecycline, IPM: Imipenem, AN: Amikacin, LEV: Levofloxacin, GM: Gentamicin, FEP: Cefepime, CAZ: Ceftazidime, CIP: Ciprofloxacin, TZP: Piperacillin/Tazobactem.

Table (3-8A, B). The result of MIC/ AST VITEK@2 GN Cards for *pseudomonas aeruginosa* isolates. (A) High resistance (B) Highly sensitive.

(A). The result of the isolation of the highly resistant *pseudomonas aeruginosa*.

Organism Quantity:
Selected Organism : Pseudomonas aeruginosa

Source: **Collected:**

Comments:

Susceptibility Information	Analysis Time: 16.08 hours			Status: Final	
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
ESBL			Imipenem	>= 16	R
Ampicillin			Amikacin	>= 64	R
Piperacillin/Tazobactam	32	S	Gentamicin	>= 16	R
Cefazolin	>= 64	R	Ciprofloxacin	>= 4	R
Cefoxitin			Levofloxacin	>= 8	R
Ceftazidime	>= 64	R	Tigecycline	>= 8	R
Ceftriaxone			Nitrofurantoin		
Cefepime	32	R	Trimethoprim/Sulfamethoxazole		
Ertapenem					

+= Deduced drug *= AES modified **= User modified

(B). The result of the isolation of the highly sensitive *pseudomonas aeruginosa.*

Organism Quantity:
Selected Organism : Pseudomonas aeruginosa

Source: **Collected:**

Comments:

Susceptibility Information	Analysis Time: 13.85 hours			Status: Final	
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
ESBL			Imipenem	2	S
Ampicillin			Amikacin	<= 2	S
Piperacillin/Tazobactam	8	S	Gentamicin	2	S
Cefazolin	>= 64	R	Ciprofloxacin	<= 0.25	S
Cefoxitin			Levofloxacin	0.5	S
Ceftazidime	2	S	Tigecycline	>= 8	R
Ceftriaxone			Nitrofurantoin		
Cefepime	<= 1	S	Trimethoprim/Sulfamethoxazole		
Ertapenem					

+= Deduced drug *= AES modified **= User modified

3.5. Molecular study

3.5.1. Extraction of genomic DNA

The extraction of genomic DNA was confirmed on gel electrophoresis of 50 isolates identified as *P. aeruginosa*, (figure 3-5). The concentration was 19.4-39.2 ng/ul and the purity was 1.83-1.97.The purpose of using this test was to monitoring the integrity of extracted DNA from *P. aeruginosa* isolates that used in the present study , rather than reading the crude extract by Nano drop. Kamat *et al.*

(2018) mentioned that, the quality and integrity of the obtained DNA proved the simplicity, rapidity, and sensitivity of the DNA extraction process . In another study, Boutin *et al.*(2018) noticed that DNA gel electrophoresis used in detection and quantification of *P. aeruginosa* is a precious tool to be added to the diagnostic toolbox.

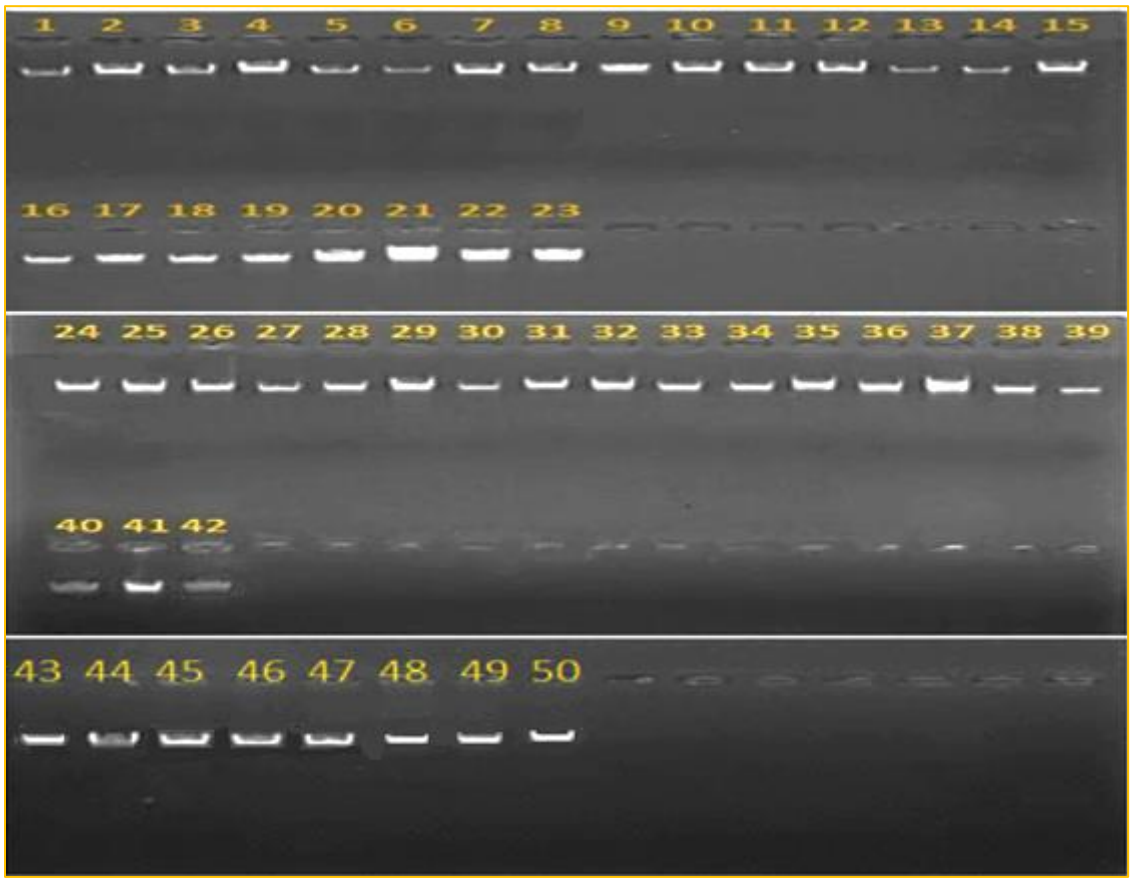


Figure (3-5). Genomic DNA Profile of *P. aeruginosa* isolates (Lanes 1-50) in 1% agarose gel Electrophoresis, for 30 min at 75 volt and 20 mA.

3.5.2. Molecular detection of *p. aeruginosa* by PCR Technique

3.5.2.1. Molecular detection of virulence factors genes.

After identification of *P. aeruginosa* by Pseudomonas chromogenic agar. As well as diagnostics using the VITEK2 device. Finally, the diagnosis was confirmed using PCR technology to detect genes (*lasI*, *lasR*, *oprI* and *oprL*) genes by using specific primers for identification *P. aeruginosa* isolates by PCR and direct sequencing to provide more accurate and reliable information.

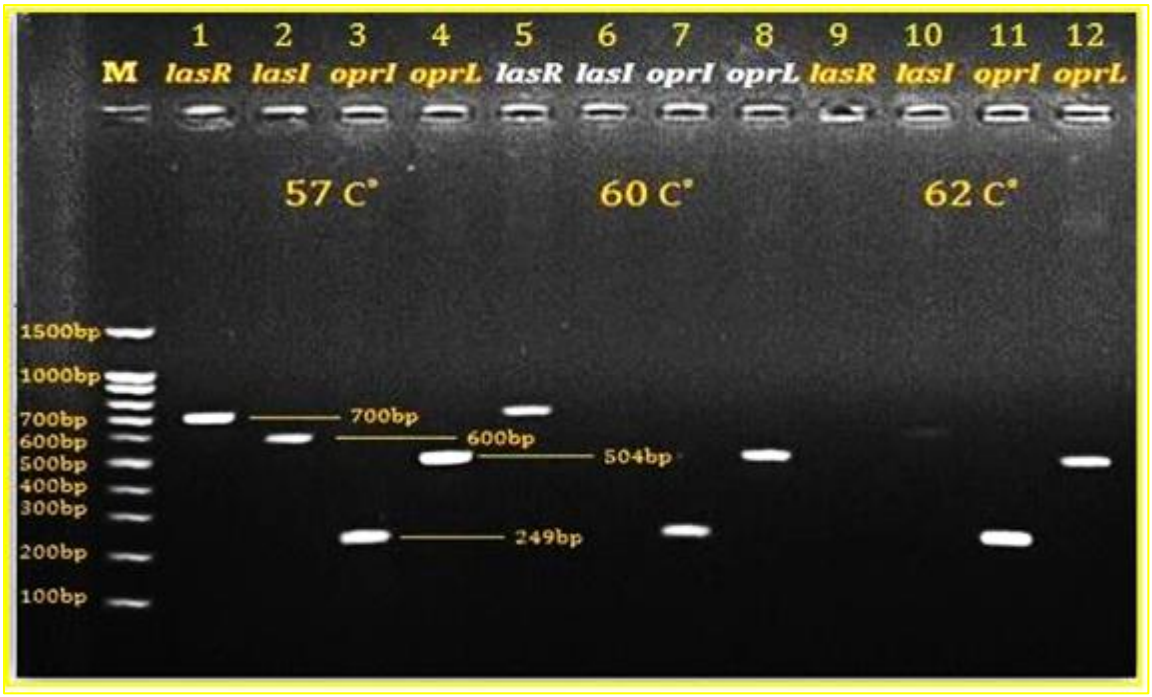


Figure (3-6).Electrophoresis patterns of gradient PCR amplification products of *lasR, lasI, oprI* and *oprL* gene of *P. aeruginosa* isolates at temperatures (57Cº, 60Cº and 62Cº) in 1.5% Agarose gel, for 45 min at 75 volt and 20 mA, (M): ladder (100-1500bp), lanes (1, 2, 3, 4): *lasR, lasI, oprI* and *oprL* genes respectively at 57Cº, lanes (5, 6, 7, 8): *lasR, lasI, oprI* and *oprL* genes at 60Cº, lanes (9, 10, 11, 12): *lasR, lasI, oprI* and *oprL* genes at 62Cº.

Figure (3-6) showed the electrophoresis patterns of *p. aeruginosa* genes (*lasR*, *lasI*, *oprI* and *oprL*) by using the gradient PCR amplification products at temperatures (57 °C, 60 °C and 62 °C), where found the best temperature is 57 °C.

Moreover, the result showed that the *lasI*, *lasR*, *oprI* and *oprL* genes were amplified from the genomic DNA of clinical isolates samples by PCR. The produced amplicons were (600bp,700bp, 249bp and 504bp) for the *lasI*, *lasR*, *oprI* and *oprL* genes, respectively. For all of the samples of *P. aeruginosa*, the PCR results were positive were positive for *lasI* as 49(98%) and *lasR* as 44(88%) and *oprI* 50(100%) and *oprL* 50(100%) (Table 3-9 and Figures 3-7,3-8,3-9 and 3-10).

Table (3-9). Presence and distribution of genes (*lasI*, *lasR*, *oprI* and *oprL*) among *P. aeruginosa* for 50 isolates

Gene	*P. aeruginosa* isolates	
	No.	%
lasI	49	(98%)
lasR	44	(88%)
oprI	50	100%
oprL	50	100%

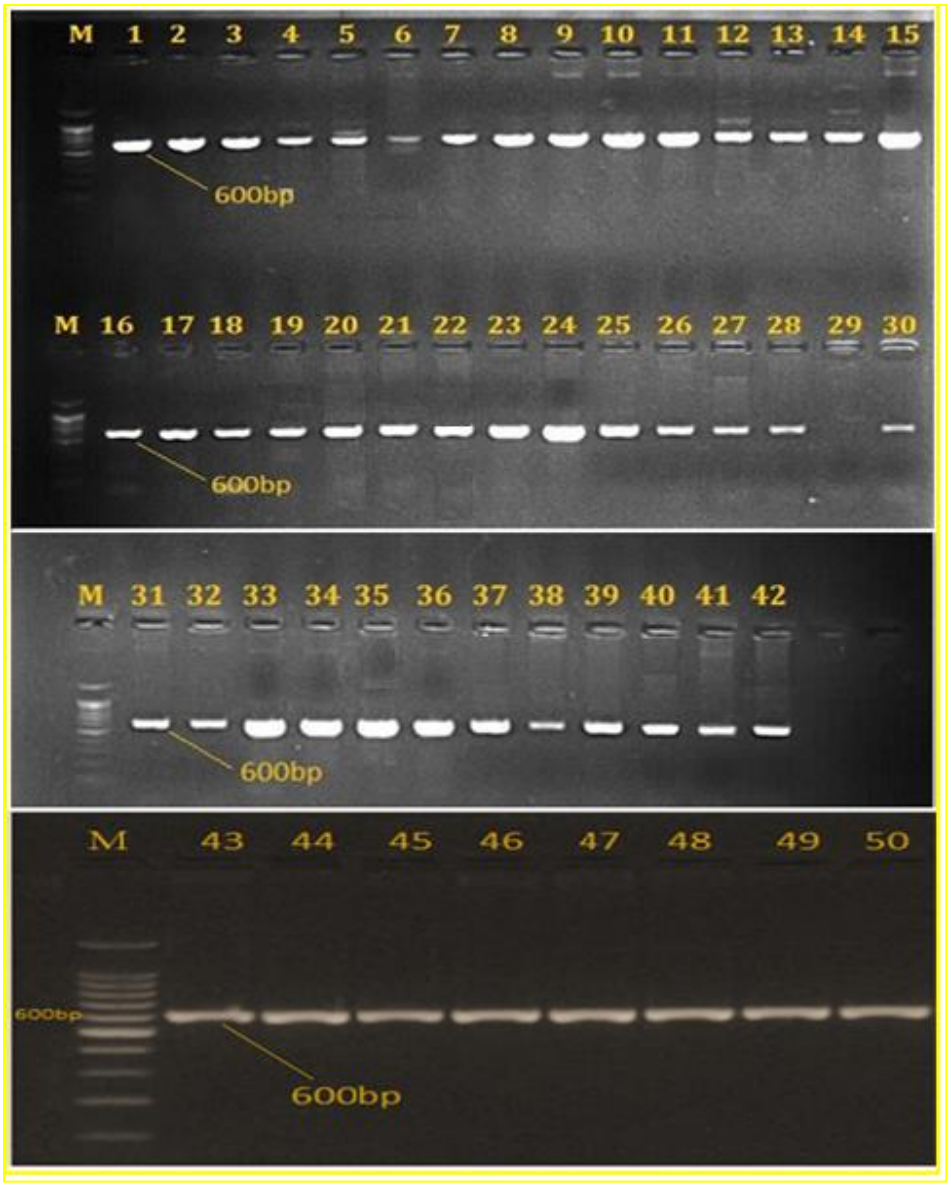


Figure (3-7). Electrophoresis patterns of PCR amplification products of *lasI* (600bp) of *P. aeruginosa* isolates in 1.5% Agarose gel, for 30 min at 75 volt and 20 MA.

Lanes M: ladder 100 -1500 bp.

Lanes (1-50) showed positive results of isolates.

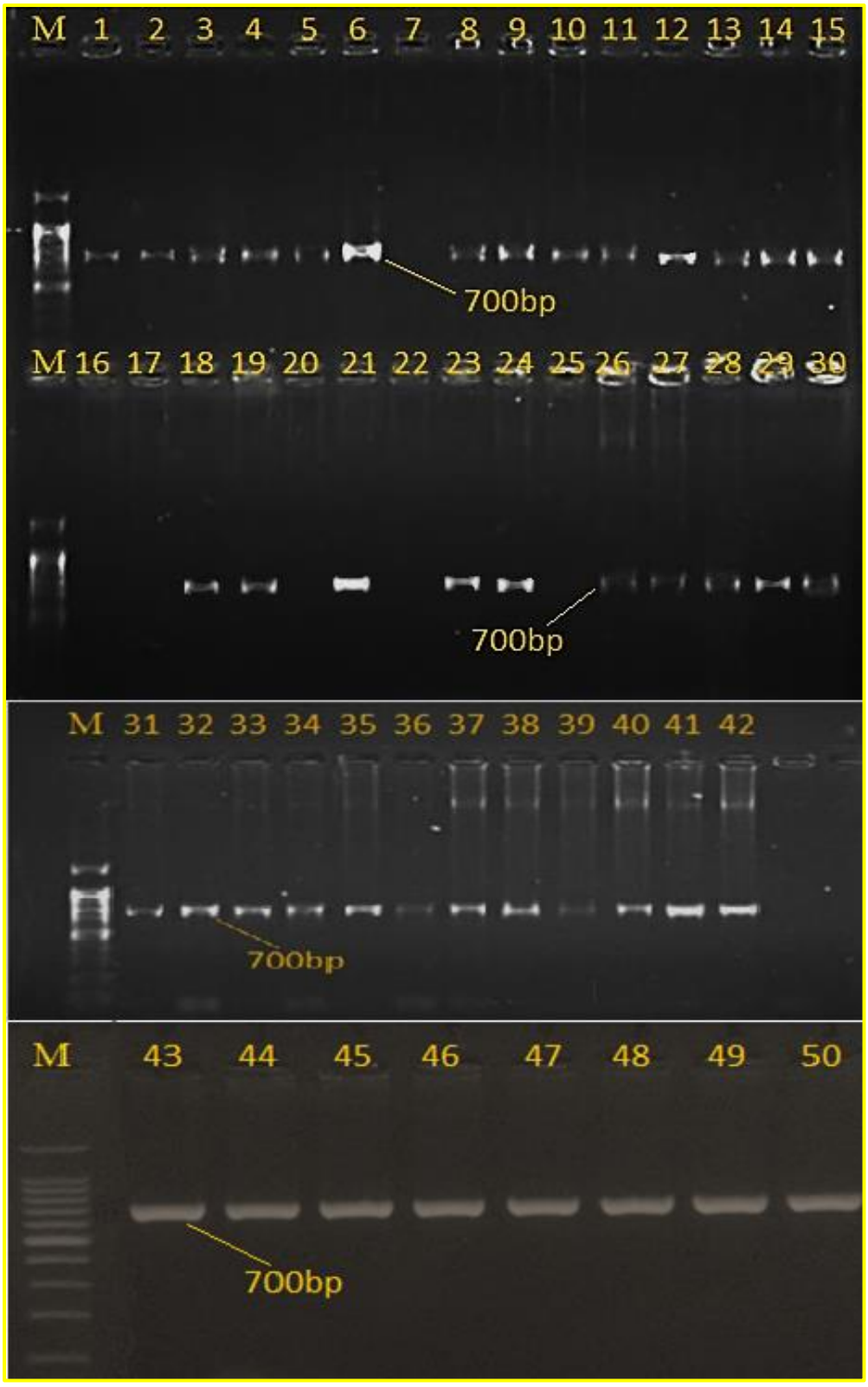


Figure (3-8). Electrophoresis patterns of PCR amplification products of *lasR* (700bp) of *P. aeruginosa* isolates in 1.5% Agarose gel, for 30 min at 75 volt and 20 mA.

Lanes M: ladder 100 -1500 bp.

Lanes (1-50): showed positive results of isolates.

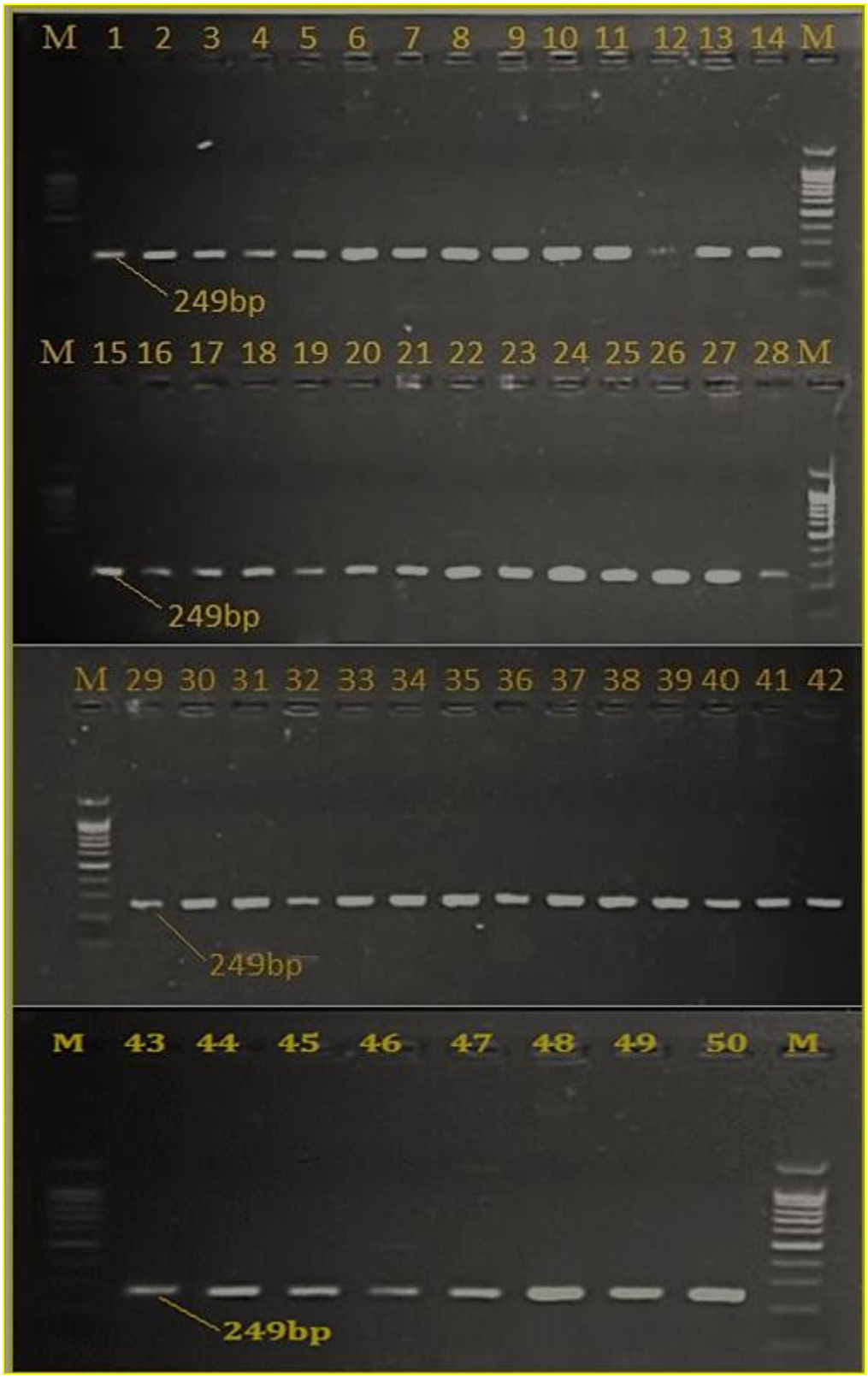


Figure (3-9). Electrophoresis patterns PCR amplification products of *oprI* (249bp) of *P. aeruginosa* isolates in 1.5% Agarose gel, for 45 min at 75 volt and 20 mA.

Lanes M : ladder 100 -1500 bp.

Lanes (1-50) showed positive results of isolates.

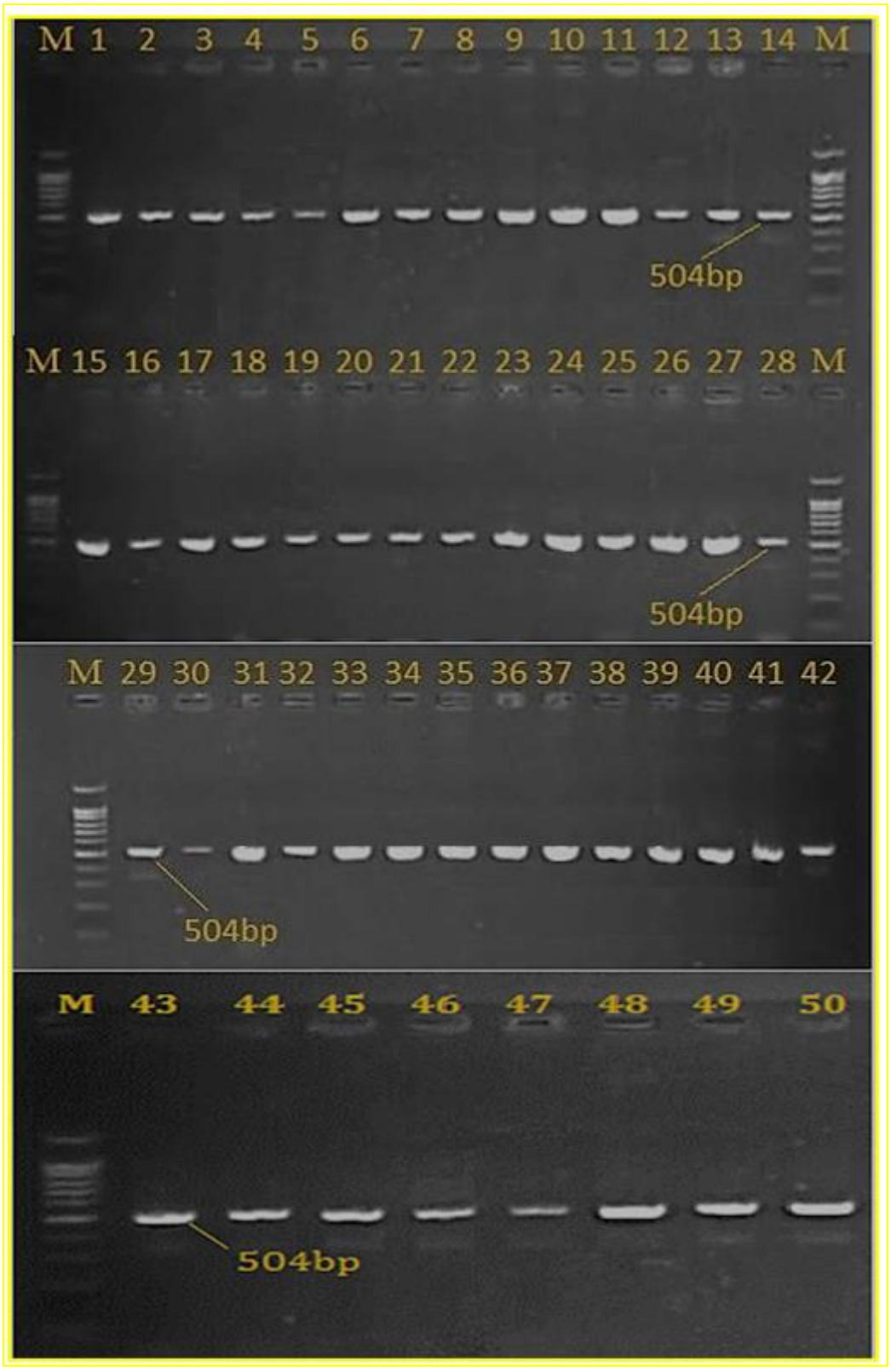


Figure (3-10). Electrophoresis patterns PCR amplification products of *oprL* (504bp) of *P. aeruginosa* isolates in 1.5% Agarose gel, for 45 min at 75 volt and 20 mA.

Lanes M : ladder 100 -1500 bp.

Lanes 1-50 showed positive results of isolates.

Regarding the *lasI* and *lasR* genes, the PCR results for the current study were positive with as percentage (98%) and (88%), respectively. these results are almost in agreement with Al-Kilabi *et al.* (2020) found that the *lasI* gene percentage was (87%), *lasR* gene (80.6%), this results come in similar with previous studies found by Salehi *et al.*(2017) and Lima *et al.*(2018) and also with Perez *et al.* (2011) who demonstrated that the detection of QS genes was high for *lasI* and *lasR* genes (81.25%). Also a study Sabharwal *et al.* (2014) and Salehi *et al.* (2017) carried out in India they found that the occurrence of *P. aeruginosa* QS genes were as follows *lasI* and *lasR* genes 75%. Also, Perez *et al.* (2013) found that the occurrence of the genes of QS was 90.1% (82/91) of the analyzed isolates presented all the genes. By contrast, the results reported by Karatuna and Yagci (2010) showed that QS genes detection were not as high. Al-Kilabi *et al.* (2020) found that quorum sensing genes (*lasI* and *lasR*) responsible for biofilm formation were present in most of the *P. aeruginosa* isolates this is good evidence that the QS system plays a critical role in the pathogenesis of *P. aeruginosa* and is an excellent way for bacteria to increase growth and resistance to antibiotics.

It is noteworthy that the early detection of the bacterium using specific genes plays a key role in control of the infection. Considering these points and given that the main virulence factors that cause the pathogenicity of *P. aeruginosa* are controlled by the quorum sensing system genes, these genes are appropriate candidate targets in PCR assays. On the other hand, several systematic studies on *P. aeruginosa* by transcriptional profiling experiments using microarrays have established that QS is a global regulatory network that controls the expression of over 300 genes by QS inducers(Smith and Iglewski, 2003).

Many studies have been developed to provide appropriate genes for the identification of *P. aeruginosa* using specific primers with high specificity. Moreover, previous studies results showed that designed primers for the *lasI* and *lasR* genes have a complementary region in all of the *P. aeruginosa* strains, and

also, have no similarity in non-*P. aeruginosa* species and other bacteria; therefore, they specifically detect only the *P .aeruginosa* strain. It should be noted that Pseudomonas species have similar quorum sensing system but the QS genes in each species are unique and conserved (Venturi, 2006).

In the current study, the presence of virulence genes encoding lipoproteins that permeate the outer membrane (*oprI* and *oprL*) of *P. aeruginosa* isolates were investigated within the genotype of bacteria the presence of which is a gain for these bacteria which enhances their resistance to environmental conditions and antibiotics, and the results of current study showed that all isolates were possessed the *oprI* and *oprL* genes with a percentage of 50(100%) within the genotype of the studied isolates.

When investigating the *oprI* and *oprL* genes encoding outer membrane proteins, the results showed that all study isolates possessed the *oprI* gene within their genotype, with a percentage of 100%, this result was consistent with Hussien *et al.* (2012) during his study on *P. aeruginosa* isolated from wounds and burns and that the sequences of this gene are highly conserved within the genetic structure of *P. aeruginosa* and most species of the genus *Fluorescent Pseudomonads* to which this bacterium belongs (De Vos *et al.*; 1997). The divergences in the distribution of virulence factor genes in the different populations might be due to the probability that some *P. aeruginosa* strains are better adopted to the particular conditions found in infectious sites that may returned to the diverse geographical and environmental sources. The prevalence of *P. aeruginosa* and its virulence genes depends on various causes consisting nature of places, degree of contamination and type, immune status of individual patients and virulence of strains (Aljebory, 2018).

The results of the detection of the *oprL* gene showed that 50 isolates with a percentage of (100 %) possess this gene within their genetic structure, this result agreed with what they obtained Khattab *et al.* (2015) during their study in which

they were used to determine the presence of the gene *oprL* in *P. aeruginosa* isolated from different clinical sources, where they found that all of the 30 isolates possessed this gene with a percentage of (100%). That the reason for its great presence in all clinical isolates and most environmental isolates is because it encodes for proteins the fatty lipoprotein which is one of the components of the outer membranes of *P. aeruginosa* bacteria especially the mature ones and in achieving the pathogenicity of many infections bacterial for being one of the virulence factors, as well as being part of the efflux pump systems for antibiotics and toxins affecting bacterial cells as it directly affects the permeability of cell membranes and thus prevents antibiotics and toxins from affecting the bacterial cell and this in turn leads to an increase in resistance (De Vos *et al*.; 1998). Also, Chand *et al.* (2020) found that the rate 87 (100%) of 87 *P. aeruginosa* isolates were positive for *oprL* genes.

As these proteins are found only in this bacteria, they could be a reliable factor for the rapid detection of *P. aeruginosa* in clinical samples (De Vos *et al*.; 1997). Also, Khattab *et al.* (2015) found that simultaneous use of (*oprI* and *oprL*) genes present more confident detection of *P. aeruginosa* by PCR.

3.6. The Sequence of Nucleotides of, *lasI*, *lasR*, *oprI* and *oprL* Genes

The knowledge of the sequence of nitrogenous bases of the outputs of the PCR reaction for 8 samples of each gene (5 sensitive and 3 resistant samples) from the *lasI*, *lasR*, *oprI*, *oprL* genes after sending 50 mL of the output of PCR for each sample with the primers of each gene to Company DNA- Macrogen/ Korea, Sequencing by Genetic Analyzer system ABI-310. After obtaining the results, all the results were compared directly with the sequences registered of global strains from different parts of the world by program of NCBI BLAST Gene Bank data base. The results were compared with the original sequence of each gene (Appendix 2A, B, C, D).

3.7. DNA Sequencing Alignment

Pseudomonas aeruginosa isolates were examined by sequencing technology to diagnosis isolates by (*lasI*, *lasR*, *oprI* and *oprL*) genes. All isolates were successful in processing of a good running of sequencing by a Company DNA-Macrogen/ Korea. The sequencing results were concordant after comparing them with data in Gene Bank/ BLAST.

3.7.1. Analysis (*lasI* and *lasR*) sequences *pseudomonas aeruginosa*

The mean of identities of the *lasI* gene was (90%) and the mean of gaps was (1%) for resistant samples, and the mean of identities was (82%) and the mean of gaps was (2.6%) for sensitive samples (Table 3- 10 and Appendix 3).

Table (3-10). DNA Sequencing Alignment/Blasts with (NCBI) of *lasI* of *P. aeruginosa* strains (PA0750, CDN118, DVT401, DVT413) chromosome analysis of gene sequencing.

No. of sample		Resistance	Sensitive	City	Burn degree	Identities	Gaps
Z1		Resistance		Najaf	Second	499/551 (91%)	2/551 (0%)
Z2			Sensitive	Najaf	Second	504/548 (92%)	7/548 (1%)
Z3			Sensitive	Babel	Second	207/259 (80%)	9/259 (3%)
Z4		Resistance		Karbala	Third	474/498 (95%)	5/498 (1%)
Z5			Sensitive	Karbala	Third	415/529 (78)	19/529 (3%)
Z6			Sensitive	Babel	Second	152/185 (82%)	4/185 (2%)
Z7		Resistance		Babel	Second	350/418 (84%)	12/418 (2%)
Z8			Sensitive	Babel	Third	244/311 (78%)	13/311 (4%)
Total Mean	Identities	90%	82%				
	Gaps	1%	2.6%				

The mean of identities of the *lasR* gene was (94.7%) and the mean of gaps was (0.33%) for resistant samples, and the mean of Identities was (92%) and the mean of gaps was (1%) for sensitive samples, except for number sample (Y2,Y7, Y8) that did not match with data in Gene Bank/ BLAST. (Table 3-11 and Appendix4).

Table (3-11). DNA Sequencing Alignment/Blasts with (NCBI) of *lasR* of *P. aeruginosa* strains (ST773, PA_154197, PA34, DVT414) chromosome Analysis of Gene Sequencing.

No. of sample		Resistance	Sensitive	City	Burn degree	Identities	Gaps
Y1			Sensitive	Najaf	Second	620/656 (95%)	8/656 (1%)
Y3			Sensitive	Babel	Second	603/676 (89%)	7/676 (1%)
Y4		Resistance		Najaf	Third	663/665 (99%)	0/665 (0%)
Y5		Resistance		Babel	Second	624/675 (92%)	11/675 (1%)
Y6		Resistance		Karbala	Third	612/661 (93%)	6/661 (0%)
Total Mean	Identities	94.7	92				
	Gaps	0.33	1				

The results of the current study for the (*lasI* and *lasR*) genes showed a high identity rate and few gaps when compared to the global strain, and this is consistent with a study conducted by Jawad *et al.* (2017), that found a high identity rate and low gaps for (*lasI* and *lasR*) genes when comparing its strains with the global strains, due to the presence of the mutations found in *lasI* and *lasR* genes of *P. aeruginosa*.

3.7.2. Analysis (*oprI* and *oprL*) sequences *pseudomonas aeruginosa*

The mean of identities of the *oprI* gene was (98%) and the mean of gaps was (0%) for resistant samples, and the mean of identities was (94%) and the mean of gaps was (1.8%) for sensitive samples (Table 3-12 and Appendix 5).

Table (3-12). DNA Sequencing Alignment/Blasts with (NCBI) of *oprI* of *P. aeruginosa* strains (DVT401, DVT779, DVT414, 2293E, UFP2) chromosome analysis of gene sequencing.

No. of sample		Resistance	Sensitive	City	Burn degree	Identities	Gaps
D1			Sensitive	Najaf	Second	213/218 (98%)	1/218 (0%)
D2			Sensitive	Babel	Second	191/193 (99%)	1/193 (0%)
D3			Sensitive	Karbala	Third	181/194 (93%)	1/194 (0%)
D4			Sensitive	Babel	Second	214/216 (99%)	1/216 (0%)
D5		Resistance		Babel	Third	207/215 (96%)	2/215 (0%)
D6		Resistance		Babel	Third	30/30 (100%)	0/30 (0%)
D7		Resistance		Babel	Third	191/194 (98%)	1/194 (0%)
D8			Sensitive	Babel	Third	138/170 (81%)	16/170 (9%)
Total Mean	Identities	98%	94%				
	Gaps	0%	1.8%				

The mean of identities of the *oprL* gene was (99%) and the mean of gaps was (0%) for resistant samples, and the mean of identities was (98.4%) and the mean of gaps was (0.2%) for sensitive samples except for number sample (W7) that did not match with data in Gene Bank/ BLAST (Table 3-13 and Appendix 6).

Table (3-13). DNA Sequencing Alignment/Blasts with (NCBI) of *oprL* of *P. aeruginosa* strains (PSE6684, PA0750, LIUYANG-A, DVT410, DVT401, ATCC 15692, DVT417) chromosome analysis of gene sequencing.

No. of sample		Resistance	Sensitive	City	Burn degree	Identities	Gaps
W1			Sensitive	Najaf	Second	453/461 (98%)	3/461 (0%)
W2			Sensitive	Babel	Second	459/467 (98%)	2/467 (0%)
W3			Sensitive	Karbala	Third	452/463 (98%)	5/463 (1%)
W4			Sensitive	Babel	Second	458/460 (99%)	1/460 (0%)
W5		Resistance		Babel	Second	465/471 (99%)	3/471 (0%)
W6			Sensitive	Babel	Third	438/442 (99%)	3/442 (0%)
W8		Resistance		Babel	Third	456/461 (99%)	4/461 (0%)
Total Mean	Identities	99	98.4				
	Gaps	0	0.2				

The results of the current study for the (*oprI* and *oprL*) genes showed a high identity rate and few gaps when compared to the global strain, and this is consistent with a study conducted by Mohammed and Ahmad, (2017) that found a high identity rate and low gaps for (*oprI* and *oprL*) genes when comparing its strains with the global strains the query results of of *oprI* gene were 99% identical to the nucleotide sequence *P. aeruginosa oprI* covering 96% DNA region. And *oprL* gene were 99% identical to the nucleotide sequence *P. aeruginosa oprL* covering 93%. The degree of similarity between the nucleotide sequence of *oprI*, *oprL* genes and *P. aeruginosa* was 99% but not 100% due to the presence of the mutations found in *oprI and oprL genes of P. aeruginosa*.

3.8. Determine the type of mutations and percentage in (*lasI*, *lasR*, *oprI* and *oprL*) genes

Microbial genomes are subject to variability due to mutation or the sequence variability within particular genes can be used in molecular typing schemes to

determine the relatedness of bacteria. An increasing number of truly complete bacterial genomes are being placed in the International Nucleotide Sequence Database Collaboration, a public database which can be searched on the web http://www.ncbi.nlm.nih.gov/sites/entrez?db=genome). This method is also acquiring practical implications for the identification and typing of microorganisms (Diancourt *et al.*, 2005; Roetzer *et al.*, 2013).

3.8.1. The type mutation and percentage in *lasI* and *lasR* genes

Showed the result of gene sequence analysis of *lasI* (Table 3-14) summarized, that there were 454(100%) mutation in 8 samples (5 sensitive and 3 resistance) of the gene *lasI*, with the mutations rate of sensitive samples (13.66%) and the mutation rate of resistant samples (10.56%).The genetic structure of *lasI* gene analyzed by sequencing for all samples that there were 260(57.3%) transversion mutations, 123(27.1%) transition mutations, 39(8.6%) insertion mutations and 32(7%) deletion mutations.

Table (3-14). Type of mutation in the *lasI* gene sequence in *p. aeruginosa* bacteria isolates.

NO. of Sample	City	Cause of Burn	Burn Degree	Antibiotic Susceptibility	Type Mutation				Total
					Transversion	Transition	Insertion	Deletion	
Z1	Najaf	Fire	Second	Resistance	37 (71.2%)	13 (25%)	2 (3.8%)	0	52 (11.4%)
Z2	Najaf	Fire	Second	Sensitive	24 (54.6%)	13 (29.5%)	5 (11.4%)	2 (4.5%)	44 (9.7%)
Z3	Babel	Fire	Second	Sensitive	31 (59.6%)	12 (23%)	3 (5.8%)	6 (11.6%)	52 (11.4%)
Z4	Karbala	liquid	Third	Resistance	11 (45.8%)	8 (33.3%)	4 (16.7%)	1 (4.2%)	24 (5.3%)
Z5	Karbala	liquid	Third	Sensitive	63 (55.3%)	32 (28%)	9 (7.9%)	10(8.8%)	114 (25.1%)
Z6	Babel	Fire	Second	Sensitive	22 (66.7%)	7 (21.2%)	3 (9.1%)	1 (3%)	33 (7.3%)
Z7	Babel	Fire	Second	Resistance	34 (50%)	22 (32.4%)	6 (8.8%)	6 (8.8%)	68 (15%)
Z8	Babel	Fire	Third	Sensitive	38 (56.7%)	16 (23.9%)	7 (10.4%)	6 (9%)	67 (14.8%)
Total summation					260 (57.3%)	123 (27.1%)	39 (8.6%)	32 (7%)	454 (100%)

Mean R =10.56% Mean S =13.66% χ^2 = 1.460 P. value = 0.692

The result of gene sequence analysis of *lasR* (Table 3-15) summarized, that there were 211(100%) mutation in 5 samples (2 sensitive and 3 resistance) of the gene *lasR*, with the mutations rate of sensitive samples (25.85%) and the mutation rate of resistant samples (16.1%).The genetic structure of *lasR* gene analyzed by sequencing for all samples that there were 107(50.7%) transversion mutations,

72(34.1%) transition mutations, 24(11.4%) insertion mutations and 8(3.8%) deletion mutations. (Appendix 7 and 8)

There was more than one mutation in each sample, and this revealed that the type and location of mutations could lead to a difference in the effect of these mutations and some of these mutations, leading to changes in the genetic code, and then a change in the amino acids at the translation level. The results of the current study of (*lasI* and *lasR*) genes agreed with a previous study of Jawad *et al.*(2017).

Table (3-15). Type of mutation in the *lasR* gene sequence in *p. aeruginosa* bacteria isolates.

NO. of Sample	City	Cause of Burn	Burn Degree	Antibiotic Susceptibility	Type Mutation				Total
					Transversion	Transition	Insertion	Deletion	
Y1	Najaf	Fire	Second	Sensitive	19 (52.8%)	9 (25%)	7 (19.4%)	1 (2.8%)	36 (17.1%)
Y3	Babel	Fire	Second	Sensitive	39 (53.4%)	27 (37%)	3 (4.1%)	4 (5.5%)	73 (34.6%)
Y4	Najaf	Fire	Third	Resistance	1 (50%)	1 (50%)	0	0	2 (0.9%)
Y5	Babel	liquid	Second	Resistance	25 (49%)	15 (29.4%)	10 (19.6%)	1 (2%)	51 (24.2%)
Y6	Karbala	liquid	Third	Resistance	23 (47%)	20 (40.8%)	4 (8.2%)	2 (4%)	49 (23.2%)
Total summation					107 (50.7%)	72 (34.1%)	24 (11.4%)	8 (3.8%)	211 (100%)

Mean R =16.1 Mean S =25.85 χ^2 = 1.752 P. value = 0.625

3.8.2. The type of mutation and percentage in *oprI* and *oprL* genes

The result of gene sequence analysis *oprI* was showed in table (3-16) that summarized, that there were 65(100%) mutation in 7 samples (5 sensitive and 2 resistance) of the gene *oprI* , with mutations rate of sensitive samples (16.62%) and the mutation rate of resistant samples (8.45%). The genetic structure of *oprI* gene analyzed by sequencing for all samples were 18(27.7%) transversion mutations, 24(36.9%) transition mutations, 22(33.9%) insertion mutations and 1(1.5%) deletion mutations.

Table (3-16). Type of mutation in the *oprI* gene sequence in *p. aeruginosa* bacteria isolates.

NO. of Sample	City	Cause of Burn	Burn Degree	Antibiotic Susceptibility	Type Mutation				Total
					Transversion	Transition	Insertion	Deletion	
D1	Najaf	Fire	Second	Sensitive	1 (20%)	3 (60%)	1 (20%)	0	5 (7.7%)
D2	Babel	Fire	Second	Sensitive	1 (50%)	0	1 (50%)	0	2 (3.1%)
D3	Karbala	liquid	Third	Sensitive	7 (53.8%)	5 (38.5%)	1 (7.7%)	0	13 (20%)
D4	Babel	Fire	Second	Sensitive	0	1 (50%)	1 (50%)	0	2 (3.1%)
D5	Babel	liquid	Third	Resistance	3 (37.5%)	3 (37.5%)	2 (25%)	0	8 (12.3%)
D7	Babel	liquid	Third	Resistance	1(33.3%)	1 (33.3%)	1 (33.4%)	0	3 (4.6%)
D8	Babel	Fire	Third	Sensitive	5 (15.6%)	11 (34.4%)	15 (46.9%)	1 (3.1%)	32 (49.2%)
Total summation					18 (27.7%)	24 (36.9%)	22 (33.9%)	1 (1.5%)	65 (100%)

Mean R = 8.45% Mean S = 16.62% χ^2 = 0.592 P. value = 0.898

The result of gene sequence analysis *oprL* was showed in table (3-17) that summarized, there were 44(100%) mutation in 7 samples (5 sensitive and 2 resistance) of the gene *oprL* , with mutations rate of sensitive samples (15%) and the mutation rate of resistant samples (12.5%). The genetic structure of *oprL* gene analyzed by sequencing for all samples that there were 17(38.7%) transversion mutations, 6(13.6%) transition mutations, 8(18.2%) insertion mutations and 13(29.5%) deletion mutations. and show that there is more than one mutation in each sample, this revealed that the type and location of mutations that were found could lead to a difference in the effect of these mutations on the genetic code, and then a change in the amino acids at the translation level (Appendix 9 and 10). The high rate of mutations in some virulence genes leads to an increase in the sensitivity of bacteria to antibiotics, as is the case in the *mexR* gene, while the percentage of mutations in other genes such as the *gyrA* gene of *P. aeruginosa* may be decreased (Gorgani *et al.*; 2009)**.**

Table (3-17). Type of mutation in the *oprL* gene sequence in p. *aeruginosa* bacteria isolates.

NO. of Sample	City	Cause of Burn	Burn Degree	Antibiotic Susceptibility	Type Mutation				Total
					Transversion	Transition	Insertion	Deletion	
W1	Najaf	Fire	Second	Sensitive	3 (37.5%)	2 (25%)	2 (25%)	1 (12.5%)	8 (18.2%)
W2	Babel	Fire	Second	Sensitive	5 (62.5%)	1 (12.5%)	1 (12.5%)	1 (12.5%)	8 (18.2%)
W3	Karbala	liquid	Third	Sensitive	6 (54.6%)	0	1 (9.1%)	4 (36.3%)	11 (25%)
W4	Babel	Fire	Second	Sensitive	1 (50%)	0	0	1 (50%)	2 (4.5%)
W5	Babel	Fire	Second	Resistance	2 (33.3%)	1 (16.7%)	2 (33.3%)	1 (16.7%)	6 (13.6%)
W6	Babel	Fire	Third	Sensitive	0	1 (25%)	1 (25%)	2 (50%)	4 (9.1%)
W8	Babel	Fire	Third	Resistance	0	1 (20%)	1 (20%)	3 (60%)	5 (11.4%)
Total summation					17 (38.7%)	6 (13.6%)	8 (18.2%)	13 (29.5%)	44 (100%)

Mean R =12.5%, Mean S =15% χ^2 = 1.471 P. value = 0.689

3.9. Phylogenic tree analysis

Drawing the comprehensive phylogenetic tree to know the evolutionary history of (*lasI*, *lasR*, *oprI*, *oprL*) genes. The genetic tree of the gene was analyzed using the program (https://mafft.cbrc.jp/alignment/server/) and it was compared with global isolates of bacteria *P. aeruginosa*.

3.9.1. Phylogenic tree analysis for *lasI* and *lasR* genes

The current genetic tree consisted of only one type of *P. aeruginosa* bacteria and it was found that all local sequences did not deviate from the type even if their mutations increased.

The results of the genetic tree of the gene *lasI* for all *P. aeruginosa* isolates when compared with the site NCBI of global isolates of *P .aeruginosa* showed that the sequences of local isolates of *P. aeruginosa* it occupied various positions within the genetic tree, and all the strains for samples (Z1, Z2, Z3, Z5, Z6, Z7, Z8) did not lie at the same level with the location of strain NCBI except for the strain for sample (Z4) located at the same level with strain NCBI, (Figure 3-11).

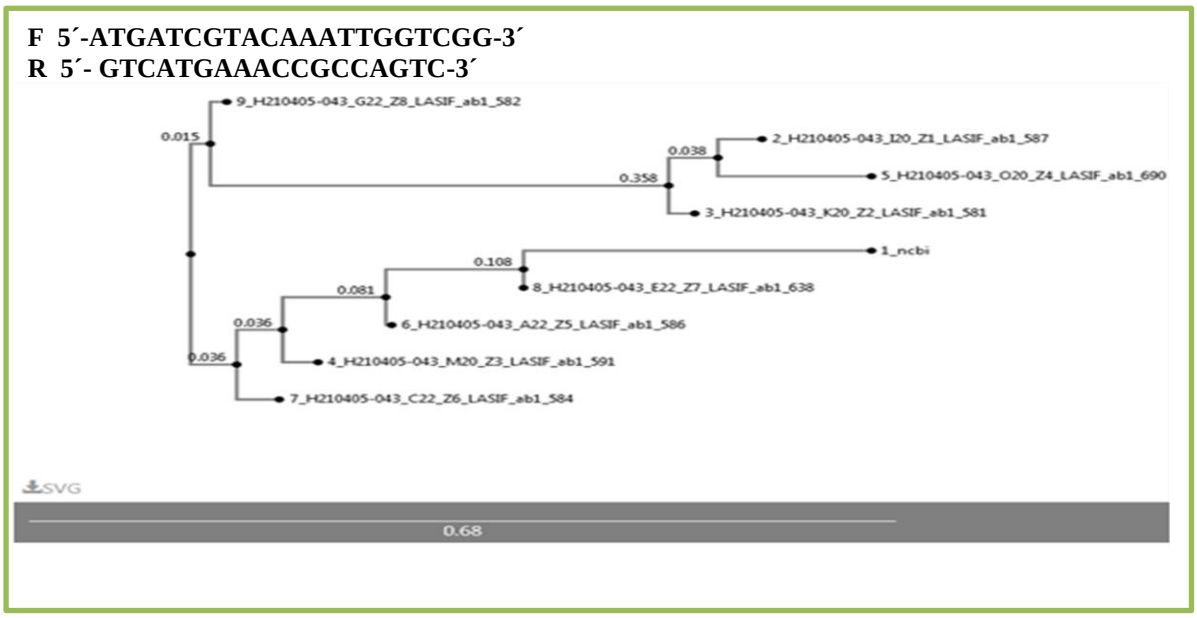


Figure (3-11). Phylogenic dendrogram *lasI* representing genetic relation between (8) isolates of *P. aeruginosa* based on (https://mafft.cbrc.jp/alignment/server/)

The results of the genetic tree of the *lasR* gene for all *P. aeruginosa* isolates when compared to the NCBI site for global isolates of *P. aeruginosa* showed the sequences of local isolates of *P. aeruginosa* within genetic tree for all sample strains (Y1, Y2, Y3, Y5, Z6, Z7, Z8) occurred at the same level with strain NCBI, (Figure 3-12). Abdullah (2019), found that the local *P. aeruginosa* sequences occupied several positions when compared with the global strains while analyzing the genetic tree, and this is due to the large number of mutations, and that the local sequences did not deviate from the type even if the number of mutations increased.

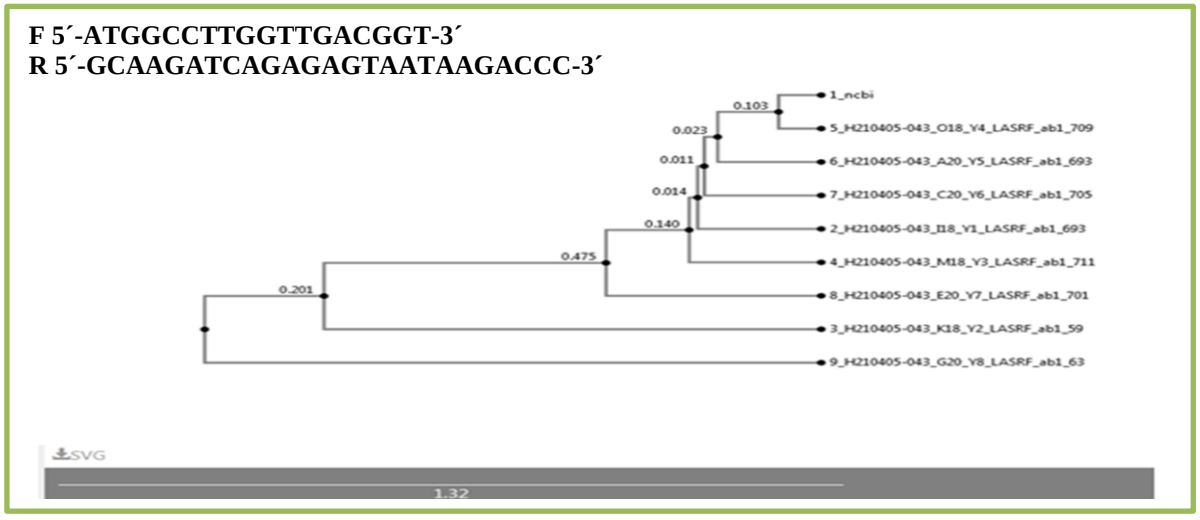


Figure (3-12). Phylogenic dendrogram *lasR* representing genetic relation between 8 isolate of *P. aeruginosa* based on (https://mafft.cbrc.jp/alignment/server/)

3.9.2. Phylogenic tree analysis for *oprI* and *oprL* genes

The current genetic tree consisted of only one type of *P. aeruginosa* bacteria and it was found that all local sequences did not deviate from the type even if their mutations increased. The results of the genetic tree of the gene *oprI* for all *P. aeruginosa* isolates when compared with the site NCBI of global isolates of *P. aeruginosa* showed that the sequences of local isolates of *P. aeruginosa* it occupied various positions within the genetic tree, 6 strains of samples (D1, D3, D5, D7) lie at the same level nearly with the location of strain NCBI except 3 strains for the of samples (D3, D6, D8) did not lie at the same level with the location of strain NCBI (Figure 3-13).

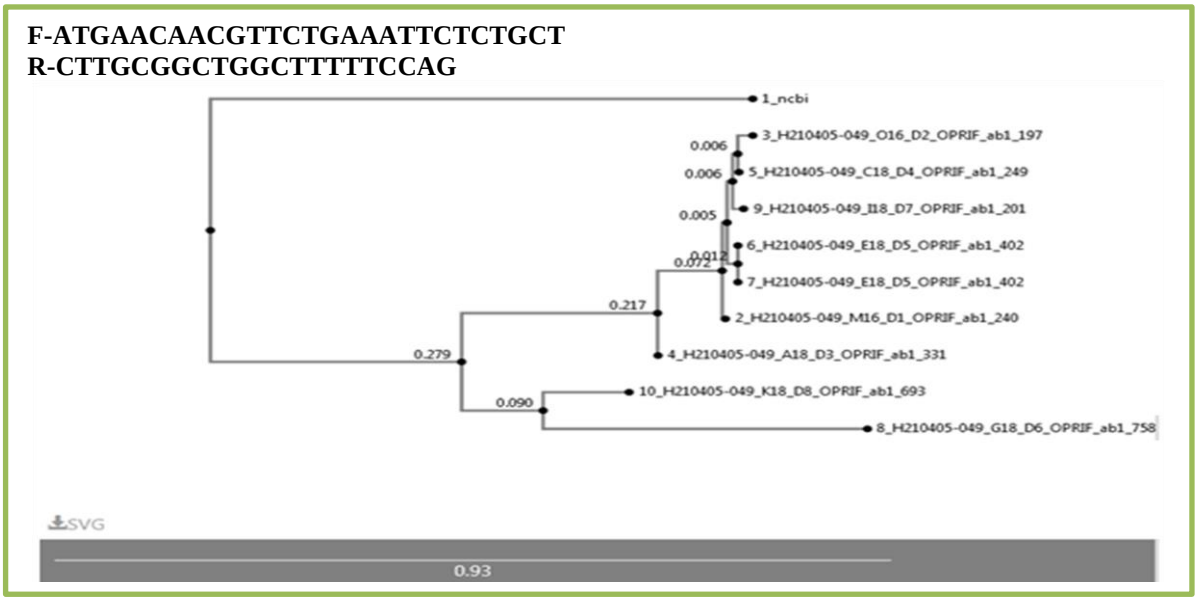


Figure (3-13). Phylogenic dendrogram *oprI* representing genetic relation between 8 isolate of *P. aeruginosa* based on (https://mafft.cbrc.jp/alignment/server/)

The results of the genetic tree of the *oprL* gene for all *P. aeruginosa* isolates when compared to the NCBI site for global isolates of *P. aeruginosa* showed the sequences of local isolates of *P. aeruginosa* within genetic tree for all sample strains (W1, W2, W3, W4, W5, W6, W7, W8) occurred at the same level with strain NCBI, as the showed in (Figure 3-14). In a study conducted by Shehab *et al.* (2020) it was found that the reason for the difference between the levels of Iraqi and international isolates may be due to mutations that caused genetic variation in the content of nucleotides.

The findings of Abdullah (2019), found that the local *P. aeruginosa* sequences occupied several positions when compared with the global strains while analyzing the genetic tree, and this is due to the large number of mutations, and that the local sequences did not deviate from the type even if the number of mutations increased. Fadhel and Hamim (2020) found that the phylogenetic tree of *oprL* gene, all phylogenetically analyzed isolates, no deviation from *P.aeruginosa* species and *oprI* and *oprL*-based comprehensive tree is an additional comprehensive tool on the high ability of the *oprL* fragment to efficiently identify *P. aeruginosa* isolates.

Mulet *et al.* (2010) found that the concatenated *oprI* and *oprL* gene tree is more congruent with the MLSA gene tree than the 16S rRNA tree. Shehab *et al.* (2020) found that the displayed the divergence between the Iraqi and global isolates might be due to the mutations that caused genetic variation in nucleotides content.

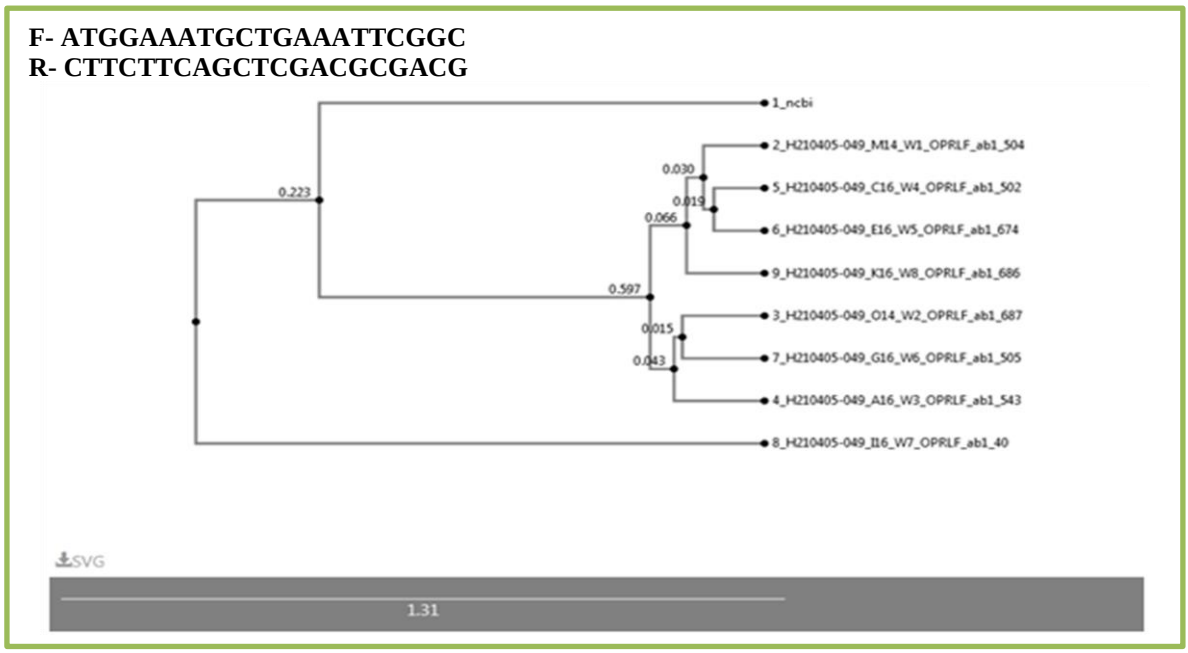


Figure (3-14).Phylogenic dendrogram *oprL* representing genetic relation between 8 isolate of *P.* aeruginosa based on (https://mafft.cbrc.jp/alignment/server/)

3.10. Three dimensional models of the *P. aeruginosa* for *lasI*, *lasR*, *oprI*, *oprL* preteins

3.10.1. Three dimensional models of the *P. aeruginosa* for *oprI* and *oprL* proteins

Figure (3-15 and 3-16) showed three dimensional models of the *P. aeruginosa* isolates (W1,W2,W4,W5,W8) for *oprL* protein and *P. aeruginosa* isolates (D2, D5) for *oprI* protein compared with the protein Data Bank (PDB), the results showed changes in DNA from sequencing that affected the quality and sequence of amino acids and thus reflected on the three dimensional protein, as the hypothetical protein structure (insilico) showed the presence of gaps at the end of the C-terminal and the transformation of β-sheet proteins due to some genetic

changes in the genes of *oprI* and *oprL*. Lima *et al.*(2018) found that the Three-dimensional modeling demonstrated that the insertion of the amino acid into the protein sequence of the *oprL* and *oprI* proteins generated a change in the protein structure reducing the antiparallel beta sheets and altering the structure of the alpha cleavage.

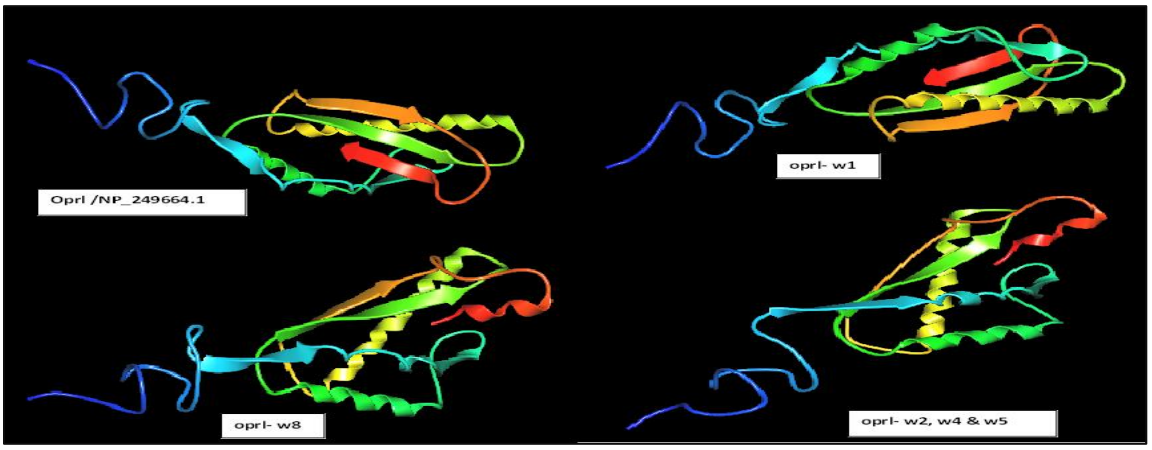


Figure (3-15). Three dimensional models of the *P. aeruginosa* of *oprL* proteins for (W1,W2,W4,W5,W8) isolates.

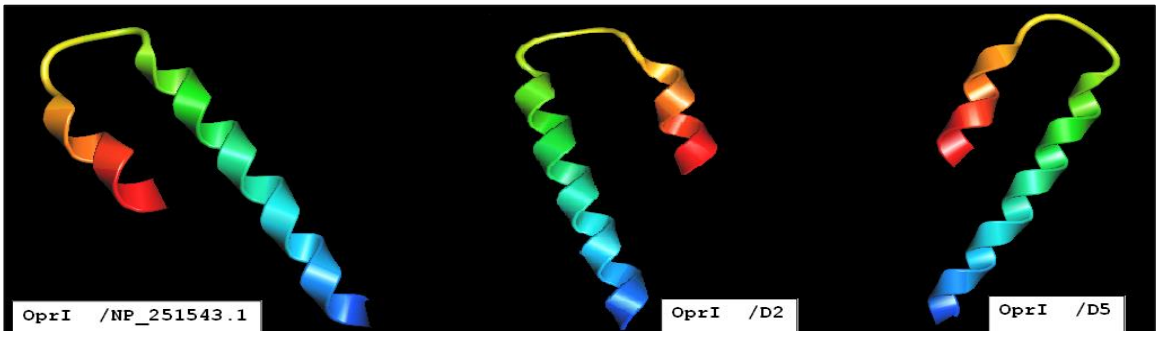


Figure (3-16). Three dimensional models of the *P .aeruginosa* of *oprI* proteins for (D2, ,D5,) isolates.

3.11. Interleukin-8 Level

The current study revealed that the concentration of IL8 in burn patients group were have significant (increase) in serum level of IL-8 between Patient and control at $P \leq 0.001$. There is significant (increase) in serum level of IL-8 between degree 2 burn patients and control and there is significant difference (increase) in serum level of IL-8 between degree 3 burn patients and control, additionally the

concentration of IL8 showed a highly elevation in degree 3 burn patients (table 3-18).

Table (3-18). Mean of IL-8 concentration (pg/ml) of burned patient(degree2 and degree 3) and control group.

Group	Mean ± SD	Sig. Value
Control (n=28)	33.308±25.790	
Degree 2 burn (n=18)	80.051±58.373	0.000276*
Degree 3 burn (n=18)	116.594±108.769	0.000159*
Patient (n=36)	98.322±88.004	0.000178*

* Significant. P ≤ (0.05) Note. 6 hemolysis samples were removed

The current study agrees with other studies that approved the elevation in serum level IL8 between burned group and control group, expression of IL-8 has been shown to be greater in burn patients (Yeh *et al.*, 1997; Finnerty *et al.*, 2007, 2013; Jeschke *et al.*, 2014), a more recent study has shown that in 468 pediatric burn patients, concentrations of IL-8 that met or exceeded a cutoff limit of 234 pg/mL were associated with higher incidence of MOF(multiple organ failure), sepsis, and mortality (Kraft *et al.*, 2015), Compared to another study the levels of IL-6, IL-8, IL10, TNF-α, and G-CSF in non-burn controls, in burn patients were significantly higher during the observed period after burn injury (P ≤0.05) (Kim *et al.*, 2012), while in other study of 322 severely burned children, a panel of biomarkers was identified, including burn size (El Ayadi *et al.*, 2018).One explanation for these discrepant results may be that, although an increase in cytokine concentration is correlated overall with burn size, other factors such as fever, bacterial infection, and WBC can cause a rapid increase in the cytokine concentration (Kim *et al.*, 2012).Table (3-19).

Table (3-19): Correlation between burn degree and interleukin-8.

		IL-8
Degree of burn	r	0.40*
	Sig.	0.023

* Correlation is significant at the 0.05 level (r = Correlation, Sig = Significant)

3.11.1. correlation of IL8 with growth of bacteria and burn degree.

The our study explained that there is significant difference (increase) in IL8 level among burned patients (degree 2 and degree 3)whom have bacterial infection and burned patients (degree 2 and degree 3) with in comparison with patients with no growth of bacteria, $P \leq 0.05$ (table 3-20).

Table (3-20) correlation of IL8 with growth of bacteria and burn degree.

Degree of burn	Growth	IL-8 concentration (pg/ml)	p-value
2	No	89.06±4.69	0.023*
	Yes	167.58±15.63	
3	No	117.09±7.31	0.011*
	Yes	255.70±14.62	

* Significant. $P \leq (0.05)$

The current study was agree with the other study that approved that IL8 was elevated in bacterial infection (Anderson *et al.*, 2019). Improved that elevation in plasma levels of sTNFR1 and IL8 consistently identified sepsis patients at higher risk of mortality and might be useful as prognostic enrichment factors in future trials by improving trial efficiency and power and reducing the number of survivors, and he Summarizing his results, when he found that IL-8 is a sensitive and specific biomarker for burn size below a threshold of 234 pg/ml and that at higher levels, the degree of plasma IL-8 correlates strongly with the incidence of septic episodes. Other study conclude that IL-8 may serve as a useful biomarker to predict infections and septic events in burn victims (Anderson *et al.*, 2019).

Other earlier study improved that, ILs, such as IL-6, that is, like IL-8, clinically used as biomarker during sepsis (Bernhard *et al.*, 2021). And other Several earlier studies highlighted the relevance of the IL-8-induced changes during systemic inflammation (Hesselink *et al.*, 2019 ; Bernhard *et al.*, 2021). However, little is known about the interaction of these parameters in an inflammatory environment created by the PAMP LPS (Bernhard *et al.*, 2021). while other consider ILs such as IL-6 and IL-8 are widely used as a diagnostic and

prognostic marker for infectious (e.g., septic) and other inflammatory (e.g., traumatic) conditions (Livaditi *et al.*, 2006; Kraft *et al.*, 2015). In the context of IL-8-induced stimulation of neutrophils (Ritter *et al.*, 1998 ; Denk *et al.*, 2017). As well as of effector functions, including ROS production (Coakley *et al.*, 2002; Behnen *et al.*, 2017), and chemotaxis (Simchowitz and Cragoe, 1986). This is making it the important biomarker in bacterial infection and sepsis.

Conclusions and Recommendations

Conclusions

The current study concludes the following:

1- The prevalence of burns in the category of children and women is more than in men and less in the elderly. The most common causes of burns are liquid burns. The second-degree burns were prevalent at all ages.

1- It was observed that *P. aeruginosa* was the dominant and most prevalent type in burn victims compared to other Gram-negative bacterial species and Pseudomonas chromogenic agar medium was a very good optional medium for the growth of this bacteria, and can be used instead of VITEK system

2- The antibiotic Piperacillin / Tazobactem was the most highly sensitive towards all isolated samples in the current study while the antibiotic Cfazolin was a resistance for all isolated samples.

3- The molecular technique of PCR provided a sensitive and rapid analytical tool for studying specific pathogens, including new emergent strains.

4- can be considered *lasI*, *lasR*, *oprI* and *oprL* genes as a successful and rapid alternative for diagnosing *P. aeruginosa* by molecular methods based on PCR and DNA sequencing technology as a result of the high presence of this genes among most of the isolates sources.

5- Increased in the level of (IL8) among burn patients with bacterial infection compared to burn patients without bacterial infection.

Recommendations

The current study highly recommended the following:

1- It is necessary to implement programs for health education relating to prevention of burn injuries by means of broadcast flashes on mass media like television or radio, showing risk situations and teaching self-care methods in workplaces and homes together with epidemiological data about burn accidents. and sentences to call attention to prevent burn accidents.

2- Drug combination regimen must be achieved due to co-resistance to multiple agents at same time and using Piperacillin-tazobactam instead piperacillin as a drug of choice to kill bacteria and prevent possible tissue damage resulted from pyocyanin secretion.

3- To diagnose *P. aeruginosa* directly from samples, future research needed to use real-time PCR and DNA sequence techniques as the routine work in hospital laboratories.

4- Using the genotype *Ecotin*, *aprA*, *hasAP*, *toxA*, *exoS*, *exoT*, *exoY*, *estA*, *plpD*, *pldAS*, *pldB* for detection *P. aeruginosa* especially in burn infection isolates.

5- Using of interleukin and TNF levels as a biochemical marker for diagnosis the degrees of burns in forensic applications.

References

Abate, A.R., Hung, T., Sperling, R.A., Mary, P., Rotem, A., Agresti, J.J., Weiner, M.A. and Weitz, D.A. 2013. DNA sequence analysis with droplet-based microfluidics. *Lab on a Chip*, 13 (24), pp.4864-4869.

Abdullah, R.M. 2019. Molecular study of *Pseudomonas aeruginosa* resistance that isolated from wounds and burns treated with disinfectants. *University of Thi-Qar Journal of Science*, (November).

Agbenorku, P., Edusei, A. and Ankomah, J. 2011. Epidemiological study of burns in Komfo Anokye Teaching Hospital, 2006-2009. *Burns*, 37 (7), pp.1259–1264.

Aghamollaei, H., Moghaddam, M.M., Kooshki, H., Heiat, M., Mirnejad, R. and Barzi, N.S. 2015. Detection of *Pseudomonas aeruginosa* by a triplex polymerase chain reaction assay based on lasI/R and gyrB genes. *Journal of infection and public health*, 8 (4), pp.314-322.

Ahmadi, A., Janbazi, S., Laghaei, Z., Ahmadi, A., Davarinejad, O. and Heidari, M. 2006. Epidemiological study of committed self-inflicted burns admitted to the Hospitals of Kermanshah, University of Medical Sciences, Iran (2004-2005). *Journal of Fundamentals of Mental Health*, 8 (29 & 30), pp.23-35.

Al Muqati, H., Al Turaiki, A., Al Dhahri, F., Al Enazi, H. and Althemery, A. 2021. Superinfection rate among the patients treated with carbapenem versus piperacillin/tazobactam: Retrospective observational study. *Journal of Infection and Public Health*, 14 (3), pp.306-310.

Al-aali, K.Y. 2016. Microbial Profile of Burn Wound Infections in Burn Patients , Taif , Saudi Arabia. *Archives of Clinical Microbiology*, 7 (2), pp.1-9.

Al-Dahmoshi, H.O.M., Al-Khafaji, N.S., Jeyad, A.A., Shareef, H.K. and Al-Jebori, R.F. 2018. Molecular detection of some virulence traits among *pseudomonas aeruginosa* isolates, Hilla-Iraq. *Biomedical and Pharmacology Journal*, 11 (2), pp.835-842.

Aldemir, M., Kara, I.H., Girgin, S. and Güloğlu, C. 2005. Factors affecting mortality and epidemiological data in patients hospitalised with burns in Diyarbakir, Turkey. *South African Journal of Surgery*, 43 (4), pp.159-162.

Aldred, K.J., Kerns, R.J. and Osheroff, N. 2014. Mechanism of quinolone action and resistance. *Biochemistry*, 53 (10), pp.1565-1574.

Alhazmi, A. 2015. *Pseudomonas aeruginosa*-Pathogenesis and Pathogenic Mechanisms. *International Journal of Biology*, 7 (2), pp.44-67.

Aliosmanoğlu, Ç. 2011. Treatment and follow-up results of children with electrical burn who observed in burn intensive care unit. *Dicle Medical Journal / Dicle tıp Dergisi*, 38 (2), pp.170-173.

Aljebory, I.S. 2018. PCR detection of some virulence genes of *Pseudomonas aeruginosa* in Kirkuk city, Iraq. *Journal of Pharmaceutical Sciences and Research*,10 (5),pp.1068-1071.

Alkateeb, D.M., Alwazni, W.S., Alghanimi, Y.K. and Altuoma, M.A. 2016. No Title Evaluation the immune status of the burn patients infected with bacteria *pseudomonas aeroginosa* in Karbala city. *IJRDPL*, 5 (4), pp.2219-2225.

Al-kilabi, A.A.K., Al-Turaihi, T.S. and Al Mohammed, H.S. 2020. Molecular detection of Quorum sensing genes in *Pseudomonas aeruginosa* isolated from CSOM patients and their relationship to biofilm ability. *Eurasian Journal of Biosciences*, 14 (2), pp.4929-4934. Foundation for Environmental Protection and Research.

Allen, T. 2013. Particle size measurement using particle size measurement. *Springer*, pp.1-31.

Al-musawi, A.N.H. and Al-garawi, H.S.M. 2015. Study of antibiotics resistance for Enterococcus faecalis isolated from different clinical casea in Al-Diwaniyah city. *Al-Qadisiyah Journal Of Pure Science*, 20 (1), pp.13-23.

Alramahy, S.K. and Aladily, Z.F. 2017. Dissemination of some antibiotic resistance in *Pseudomonas aeruginosa* isolates in Al-Diwaniya Hospitals. *Al-Qadisiyah Journal Of Pure Science*, 22 (3), pp.349-359.

American Burn Association. 2019. National burn repository 2019 update: Report of data from 2009–2018. *National burn repository 2019 update: Report of data from 2009–2018*, 60606 (312).

Anderson, B.J., Calfee, C.S., Liu, K.D., Reilly, J.P., Kangelaris, K.N., Shashaty, M.G.S., Lazaar, A.L., Bayliffe, A.I., Gallop, R.J. and Miano, T.A. 2019. Plasma sTNFR1 and IL8 for prognostic enrichment in sepsis trials: a prospective cohort study. *Critical Care*, 23 (1), pp.1–9.

Anil, C. and Shahid, R.M. 2013. Antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* clinical isolates at a tertiary care hospital in Kathmandu, Nepal. *Asian J Pharm Clin Res*, 6 (3), pp.235-238.

Anvarinejad, M., Japoni, A., Rafaatpour, N., Mardaneh, J., Abbasi, P., Shahidi, M.A., Dehyadegari, M.A. and Alipour, E. 2014. Burn patients infected with metallo-beta-lactamase-producing *pseudomonas aeruginosa*: Multidrug-resistant strains. *Archives of Trauma Research*, 3 (2).

Apostolakis, S., Vogiatzi, K., Amanatidou, V. and Spandidos, D.A. 2009. Interleukin 8 and cardiovascular disease. *Cardiovascular Research*, 84 (3), pp.353-360.

Arber, W. 2014. Horizontal gene transfer among bacteria and its role in biological evolution. *Life*, 4 (2), pp.217-224.

Arzanlou, M., Chai, W.C. and Venter, H. 2017. Intrinsic, adaptive and acquired antimicrobial resistance in Gram-negative bacteria. *Essays in Biochemistry*, 61 (1), pp.49-59.

Azeredo, J., Azevedo, N.F., Briandet, R., Cerca, N., Coenye, T., Costa, A.R., Desvaux, M., Di Bonaventura, G., Hébraud, M., Jaglic, Z., Kačániová, M.,2017. Critical review on biofilm methods. *Critical Reviews in Microbiology*, 43 (3), pp.313-351.

Balasubramanian, D., Schneper, L., Kumari, H. and Mathee, K. 2013. A dynamic and intricate regulatory network determines *Pseudomonas aeruginosa* virulence. *Nucleic Acids Research*, 41 (1), pp.1-20.

Banar, M., Emaneini, M., Satarzadeh, M., Abdellahi, N., Beigverdi, R., Van Leeuwen, W.B. and Jabalameli, F. 2016. Evaluation of mannosidase and trypsin enzymes effects on biofilm production of *Pseudomonas aeruginosa* isolated from burn wound infections. *PloS one*, 11 (10), pp.1-13.

Baron, E.J., Finegold, S.M. and Peterson, I.L.R. 2007. Bailey and Scott's diagnostic microbiology. *American Journal of Clinical Pathology*. Mosby.

Bassetti, M., Vena, A., Croxatto, A., Righi, E. and Guery, B. 2018. How to manage *Pseudomonas aeruginosa* infections. *Drugs in Context*, 7. BioExcel Publishing Ltd.

Beale, J.M. and Block, J.H. 2011. *Wilson and Giswold's Organic medicinal and Pharmaceutical chemistry*. Wolters Kluwer India.

Behnen, M., Möller, S., Brozek, A., Klinger, M. and Laskay, T. 2017. Extracellular acidification inhibits the ROS-dependent formation of neutrophil extracellular traps. *Frontiers in immunology*, 8, p.184.

Bekele, T., Tesfaye, A., Sewunet, T. and Waktola, H.D. 2015. *Pseudomonas aeruginosa* isolates and their antimicrobial susceptibility pattern among catheterized patients at Jimma University Teaching Hospital, Jimma, Ethiopia. *BMC Research Notes*, 8 (1), pp.1-4.

Bergen, G.A. and Shelhamer, J.H. 1996. Pulmonary infiltrates in the cancer patient: New approaches to an old problem. *Infectious Disease Clinics*, 10 (2), pp.297-325.

Bergquist, M., Hästbacka, J., Glaumann, C., Freden, F., Huss, F. and Lipcsey, M. 2019. The time-course of the inflammatory response to major burn injury and its relation to organ failure and outcome. *Burns*, 45 (2), pp.354-363.

Bernhard, S., Hug, S., Stratmann, A.E.P., Erber, M., Vidoni, L., Knapp, C.L., Thomaß, B.D., Fauler, M., Nilsson, B., Nilsson Ekdahl, K., Föhr, K., 2021. Interleukin 8 elicits rapid physiological changes in neutrophils that are altered by inflammatory conditions. *Journal of Innate Immunity*, 13 (4), pp.225-241.

Berrazeg, M., Jeannot, K., Ntsogo Enguéné, V.Y., Broutin, I., Loeffert, S., Fournier, D. and Plésiat, P. 2015. Mutations in β-lactamase AmpC increase resistance of *Pseudomonas aeruginosa* isolates to antipseudomonal cephalosporins. *Antimicrobial Agents and Chemotherapy*, 59 (10), pp.6248-6255.

Beveridge, T.J. 1999. Structures of gram-negative cell walls and their derived membrane vesicles. *Journal of Bacteriology*, 181 (16), pp.4725-4733.

Bhatt, P., Rathi, K., Hazra, S., Sharma, A. and Shete, V. 2015. Prevalence of multidrug resistant *Pseudomonas aeruginosa* infection in burn patients at a tertiary care centre. *Indian Journal of Burns*, 23 (1), p.56.

Bhatta, D.R., Hamal, D., Shrestha, R., Supram, H.S., Joshi, P., Nayak, N. and Gokhale, S. 2019. Burden of multidrug resistant respiratory pathogens in intensive care units of tertiary care hospital. *Asian Journal of Medical Sciences*, 10 (2), pp.14-19.

Biccard, B.M. and Rodseth, R.N. 2011. Utility of clinical risk predictors for preoperative cardiovascular risk prediction. *British Journal of Anaesthesia*, 107 (2), pp.133-143.

Bonomo, R.A. and Szabo, D. 2006. Mechanisms of multidrug resistance in Acinetobacter species and *Pseudomonas aeruginosa*. *Clinical infectious diseases*, 43 (Supplement_2), pp.S49-S56.

Bottomley, M.J., Muraglia, E., Bazzo, R. and Carfì, A. 2007. Molecular insights into quorum sensing in the human pathogen *Pseudomonas aeruginosa* from the structure of the virulence regulator LasR bound to its autoinducer. *Journal of Biological Chemistry*, 282 (18), pp.13592-13600.

Boutin, S., Weitnauer, M., Hassel, S., Graeber, S.Y., Stahl, M., Dittrich, A.S., Mall, M.A. and Dalpke, A.H. 2018. One time quantitative PCR detection of *Pseudomonas aeruginosa* to discriminate intermittent from chronic infection in cystic fibrosis. *Journal of Cystic Fibrosis*, 17 (3), pp.348-355.

Boyer, A., Doussau, A., Thiébault, R., Venier, A.G., Tran, V., Boulestreau, H., Bébéar, C., Vargas, F., Hilbert, G. and Gruson, D. 2011. *Pseudomonas aeruginosa* acquisition on an intensive care unit: relationship between antibiotic selective pressure and patients' environment. *Critical care*, 15 (1), pp.1-10.

Brahmer, J.R., Tykodi, S.S., Chow, L.Q.M., Hwu, W.J., Topalian, S.L., Hwu, P., Drake, C.G., Camacho, L.H., Kauh, J., Odunsi, K. and Pitot, H.C., 2012. Safety and Activity of Anti–PD-L1 Antibody in Patients with Advanced Cancer. *New England Journal of Medicine*, 366 (26), pp.2455-2465.

Breidenstein, E.B.M., de la Fuente-Núñez, C. and Hancock, R.E.W. 2011.*Pseudomonas aeruginosa*: all roads lead to resistance. *Trends in microbiology*, 19 (8), pp.419-426.

Brooks, A.G.F., Carroll, K.C., Butel, J.S. and Morse, S.A. 2007. Jawetz, Melnick & Adelberg's Medical Microbiology 24 th Edition. *Sultan Qaboos University Medical*, 7 (3), pp.273-275

Brown, S.P., Cornforth, D.M. and Mideo, N. 2012. Evolution of virulence in opportunistic pathogens:Generalism, plasticity, and control. *Trends in Microbiology*,20 (7), pp.336-342.

Brugha, R.E. and Davies, J.C. 2011. *Pseudomonas aeruginosa* in cystic fibrosis: pathogenesis and new treatments. *British journal of hospital medicine*, 72 (11), pp.614-619.

Buja, Z., Arifi, H. and Hoxha, E. 2010. Electrical burn injuries. An eight-year review. *Annals of Burns and Fire Disasters*, 23 (1), pp.4-7.

Bush, K. and Jacoby, G.A. 2010. Updated functional classification of β-lactamases. *Antimicrobial agents and chemotherapy*, 54 (3), pp.969-976.

Cabot, G., Ocampo-Sosa, A.A., Tubau, F., Macia, M.D., Rodríguez, C., Moya, B., Zamorano, L., Suárez, C., Peña, C. and Martínez-Martínez, L. 2011. Overexpression of AmpC and efflux pumps in *Pseudomonas aeruginosa* isolates from bloodstream infections: prevalence and impact on resistance in a Spanish multicenter study. *Antimicrobial agents and chemotherapy*, 55 (5), pp.1906–1911.

Cabot, G., Zamorano, L., Moyà, B., Juan, C., Navas, A., Blázquez, J. and Oliver, A. 2016. Evolution of *pseudomonas aeruginosa* antimicrobial resistance and fitness under low and high mutation rates. *Antimicrobial agents and chemotherapy*, 60 (3), pp.1767-1778.

Çakir, B. and Yeğen, B.C. 2004. Systemic responses to burn injury. *Turkish Journal of Medical Sciences*, 34 (4), pp.215-226.

Cassin, E.K. and Tseng, B.S. 2019. Pushing beyond the envelope: The potential roles of oprf in *pseudomonas aeruginosa* biofilm formation and pathogenicity. *Journal of Bacteriology*, 201 (18), pp.1-12.

Castanheira, M., Toleman, M.A., Jones, R.N., Schmidt, F.J. and Walsh, T.R. 2004. Molecular characterization of a β-lactamase gene, blaGIM-1, encoding a new subclass of metallo-β-lactamase. *Antimicrobial agents and chemotherapy*, 48 (12), pp.4654-4661.

Cavalcanti, F.L. de S., Mirones, C.R., Paucar, E.R., Montes, L.Á., Leal-Balbino, T.C., Morais, M.M.C. de, Martínez-Martínez, L. and Ocampo-Sosa, A.A. 2015. Mutational and acquired carbapenem resistance mechanisms in multidrug resistant *pseudomonas aeruginosa* clinical isolates from Recife, Brazil. *Memórias do Instituto Oswaldo Cruz*, 110 (8), pp.1003-1009.

Chand, Y., Khanal, S., Panta, O.P., Shrestha, D., Khadka, D.K. and Poudel, P. 2020. Prevalence of some virulence genes and antibiotic susceptibility pattern of *Pseudomonas aeruginosa* isolated from different clinical specimens. *research square*, pp.3-17.

Chastre, J. and Fagon, J.Y. 2002. Ventilator-associated pneumonia. *American journal of respiratory and critical care medicine*, 165 (7), pp.867-903.

Chatterjee, M., Anju, C.P., Biswas, L., Anil Kumar, V., Gopi Mohan, C. and Biswas, R. 2016. Antibiotic resistance in *Pseudomonas aeruginosa* and alternative therapeutic options. *International Journal of Medical Microbiology*, 306(1), pp.48-58.

Chen, J., Su, Z., Liu, Y., Wang, S., Dai, X., Li, Y., Peng, S., Shao, Q., Zhang, H. and Wen, P. 2009. Identification and characterization of class 1 integrons among *Pseudomonas aeruginosa* isolates from patients in Zhenjiang, China. *International Journal of Infectious Diseases*, 13 (6), pp.717-721.

Chevalier, S., Bouffartigues, E., Bodilis, J., Maillot, O., Lesouhaitier, O., Feuilloley, M.G.J., Orange, N., Dufour, A. and Cornelis, P. 2017. Structure, function and regulation of *Pseudomonas aeruginosa* porins. *FEMS Microbiology Reviews*, 41 (5), pp.698-722.

Chmielecki, J. and Meyerson, M. 2014. DNA sequencing of cancer: What have we learned? *Annual Review of Medicine*, 65 (10), pp.63-79.

Chuang, L.-Y., Cheng, Y.-H. and Yang, C.-H. 2013. Specific primer design for the polymerase chain reaction. *Biotechnology Letters*, 35, pp.1541-1549.

Church, D., Elsayed, S., Reid, O., Winston, B. and Lindsay, R. 2006. Burn wound infections. *Clinical Microbiology Reviews*, 19 (2), pp.403-434.

Coakley, R.J., Taggart, C., McElvaney, N.G. and O'Neill, S.J. 2002. Cytosolic pH and the inflammatory microenvironment modulate cell death in human neutrophils after phagocytosis. *Blood*, 100 (9), pp.3383-3391.

Collee, J.G., Fraser, A.G., Marmino, B.P. and Simons, A. 1996. Mackin and McCartney Practical Medical Microbiology. The Churchill Livingstone. *Inc. USA.*

Cooke, A.C. and Schertzer, J.W. 2019. Investigations into the Biogenesis and Functions of Outer Membrane Vesicles in *Pseudomonas aeruginosa* Biofilms.*State University of New York*, pp. 196

. de Almeida Silva, K. de C.F., Calomino, M.A., Deutsch, G., de Castilho, S.R., de Paula, G.R., Esper, L.M.R. and Teixeira, L.A. 2017. Molecular characterization of multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolated in a burn center. *Burns*, 43 (1), pp.137-143.

De Bentzmann, S. and Plésiat, P. 2011. The *Pseudomonas aeruginosa* opportunistic pathogen and human infections. *Environmental Microbiology*, 13 (7), pp.1655-1665.

De Macedo, J.L.S., Rosa, S.C. and Castro, C. 2003. Sepsis in burned patients. *Revista da Sociedade Brasileira de Medicina Tropical*, 36 (6), pp.647-652.

De Vos, D., Bouton, C., Sarniguet, A., De Vos, P., Vauterin, M. and Cornelis, P. 1998. Sequence diversity of the oprI gene, coding for major outer membrane lipoprotein I, among rRNA group I pseudomonads. *Journal of Bacteriology*, 180 (24), pp.6551-6556.

De Vos, D., Lim, A., Pirnay, J.P., Struelens, M., Vandenvelde, C., Duinslaeger, L., Vanderkelen, A. and Cornelis, P. 1997. Direct detection and identification of *Pseudomonas aeruginosa* in clinical samples such as skin biopsy specimens and expectorations by multiplex PCR based on two outer membrane lipoprotein genes, *oprI*

and *oprL*. *Journal of Clinical Microbiology*, 35 (6), pp.1295-1299.

Demirdjian, S., Sanchez, H., Hopkins, D. and Berwin, B. 2019. Motility-Independent Formation of Antibiotic-Tolerant *Pseudomonas aeruginosa* Aggregates. *Applied and Environmental Microbiology*, 85 (14), pp.1-12.

Denk, S., Neher, M.D., Messerer, D.A.C., Wiegner, R., Nilsson, B., Rittirsch, D., Nilsson-Ekdahl, K., Weckbach, S., Ignatius, A., Kalbitz, M., Gebhard, F., 2017. Complement C5a Functions as a Master Switch for the pH Balance in Neutrophils Exerting Fundamental Immunometabolic Effects. *The Journal of Immunology*, 198 (12), pp.4846-4854.

Dettman, J.R., Rodrigue, N., Aaron, S.D. and Kassen, R. 2013. Evolutionary genomics of epidemic and nonepidemic strains of *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences of the United States of America*, 110 (52), pp.21065-21070.

Diancourt, L., Passet, V., Verhoef, J., Grimont, P.A.D. and Brisse, S. 2005. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *Journal of clinical microbiology*, 43 (8), pp.4178-4182.

Dong, L., Yoshizawa, J. and Li, X. 2019. Nucleic Acid Isolation and Quality Control. *Biobanking*, pp.325-343.

Dou, Y., Huan, J., Guo, F., Zhou, Z. and Shi, Y. 2017. *Pseudomonas aeruginosa* prevalence, antibiotic resistance and antimicrobial use in Chinese burn wards from 2007 to 2014. *Journal of International Medical Research*, 45 (3), pp.1124-1137.

Douraghi, M., Ghasemi, F., Soltan Dallal, M.M., Rahbar, M. and Rahimiforoushani, A. 2014. Molecular identification of *Pseudomonas aeruginosa* recovered from cystic fibrosis patients. *Journal of Preventive Medicine and Hygiene*, 55 (2), pp.50-53.

Drawz, S.M. and Bonomo, R.A. 2010. Three decades of β-lactamase inhibitors. *Clinical microbiology reviews*, 23 (1), pp.160-201.

Drenkard, E. 2003. Antimicrobial resistance of *Pseudomonas aeruginosa* biofilms. *Microbes and infection*, 5 (13), pp.1213-1219.

Driscoll, J.A., Brody, S.L. and Kollef, M.H. 2007. The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. *Drugs*, 67 (3), pp.351-368.

Duchene, M., Barron, C., Schweizer, A., Von Specht, B.U. and Domdey, H. 1989. *Pseudomonas aeruginosa* outer membrane lipoprotein I gene: Molecular cloning, sequence, and expression in *Escherichia coli*. *Journal of Bacteriology*, 171 (8), pp.4130-4137.

Dutra, V.G., Alves, V.M.N., Olendzki, A.N., Dias, C.A.G., de Bastos, A.F.A., Santos, G.O., de Amorin, E.L.T., Sousa, M.T., Santos, R., Ribeiro, P.C.S. and Fontes, C.F., 2014. *Streptococcus agalactiae* in Brazil: Serotype distribution, virulence determinants and antimicrobial susceptibility. *BMC Infectious Diseases*, 14 (1), pp.323.

Ehsani, E., Barrantes, I., Vandermaesen, J., Geffers, R., Jarek, M., Boon, N., Springael, D., Pieper, D.H. and Vilchez-Vargas, R. 2016. Draft genome sequence of Aeromonas sp. strain EERV15. *Genome Announcements*, 4 (4). pp.e00811-16.

El Ayadi, A., Herndon, D.N. and Finnerty, C.C. 2018. Biomarkers in burn patient care. In: *Total Burn Care: Fifth Edition*.pp.232-235.e2.

El Solh, A.A. and Alhajhusain, A. 2009. Update on the treatment of *Pseudomonas aeruginosa* pneumonia. *Journal of Antimicrobial Chemotherapy*, 64 (2), pp.229-238.

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

El-Mowafy, S.A., Abd El Galil, K.H., El-Messery, S.M. and Shaaban, M.I. 2014. Aspirin is an efficient inhibitor of quorum sensing, virulence and toxins in *Pseudomonas aeruginosa*. *Microbial Pathogenesis*, 74 (1), pp.25-32.

Elsous, A., Salah, M. and Ouda, M. 2015. Childhood burns: an analysis of 124 admissions in the Gaza Strip. *Annals of burns and fire disasters*, 28 (4), pp.253-258.

Essayagh, M., Essayagh, T., Essayagh, S. and El Hamzaoui, S. 2019. Epidemiology of burn wound infection in Rabat, Morocco: Three-year review. *Médecine et Santé Tropicales*, 24 (2), pp.157-164.

Fadhel, Z.A. and Hamim, S.S. 2020. Phylogenetic Analysis of *oprL* and *oprI*genes in *Pseudomonas Aeruginosa* Isolated from Otitis Media Patients. *Indian Journal of Public Health Research & Development*, 11 (4), pp.1201.

Farhood, A.H. and Chelab, R.L. 2017. Isolation and Identification of Bacteria from Burn injuries by 16S rRNA Gene. *Journal of Education for Pure Science*, 7 (1).

Fariñas, M.C. and Martínez-Martínez, L. 2013. Multiresistant Gram-negative bacterial infections: Enterobacteria, *Pseudomonas aeruginosa*, Acinetobacter baumannii and other non-fermenting Gram-negative bacilli. *Enfermedades infecciosas y microbiologia clinica*, 31 (6), pp.402-409.

Fernández, L. and Hancock, R.E.W. 2012. Adaptive and mutational resistance: Role of porins and efflux pumps in drug resistance. *Clinical Microbiology Reviews*, 25 (4), pp.661-681.

Finnerty, C.C., Herndon, D.N., Chinkes, D.L. and Jeschke, M.G. 2007. Serum cytokine differences in severely burned children with and without sepsis. *Shock*, 27 (1), pp.4-9.

Finnerty, C.C., Jeschke, M.G., Qian, W.J., Kaushal, A., Xiao, W., Liu, T., Gritsenko, M.A., Moore, R.J., Camp, D.G., 2013. Determination of burn patient outcome by large-scale quantitative discovery proteomics. *Critical Care Medicine*, 41 (6), pp.1421-1434.

Forbes, B.A., Sahm, D.F. and Weissfeld, A.S. 2002. Bailey and Scotts Diagnostic Microbiology. 11 th. *St. Louis, Mo, USA: Mosby*, pp.711-798.

Fujitani, S., Moffett, K.S. and Yu, V.L. 2017. *Pseudomonas aeruginosa*. *Antimicrobe: Infectious Disease & Antimicrobial Agents*, PA,15219.

Gayathri, R., Cauveri, A., Kanagapriya, R., Nivetha, V., Tamizhselvi, P. and Premkumar, K. 2015. A novel approach for clustering based on Bayesian network. In: *ACM International Conference Proceeding Series*. pp.1-5.

Gebel, J., Exner, M., French, G., Chartier, Y., Christiansen, B., Gemein, S., Goroncy-Bermes, P., Hartemann, P., Heudorf, U., Kramer, A. and Maillard, J.Y., 2013.The role of surface disinfection in infection prevention. *GMS hygiene and infection control*, 8 (1), pp.10.

Gellatly, S.L. and Hancock, R.E.W. 2013. *Pseudomonas aeruginosa*: New insights into pathogenesis and host defenses. *Pathogens and Disease*, 67 (3), pp.159-173.

George, E.L. and Panos, A. 2005.Does a high WBC count signal infection? *Nursing*,35 (1),pp.20-21.

George, S., Basavarajappa, K.G. and Hanumanthappa, A.R. 2015. Bacteriological study of burns infection. *Journal of Evolution of Medical and Dental Sciences*, 4 (81), pp.14216-14225.

Ghazalibina, M., Morshedi, K., Farahani, R.K., Babadi, M. and Khaledi, A. 2019. Study of virulence genes and related with biofilm formation in *Pseudomonas aeruginosa* isolated from clinical samples of Iranian patients; A systematic review. *Gene Reports*, 17, p.100471.

Gnaneswaran, N., Perera, E., Perera, M. and Sawhney, R. 2015. Cutaneous chemical burns: Assessment and early management. *Australian Family Physician*, 44 (3), pp.135-139.

Gonzalez, M.R., Fleuchot, B., Lauciello, L., Jafari, P., Applegate, L.A., Raffoul, W., Que, Y.-A. and Perron, K. 2016. Effect of human burn wound exudate on *Pseudomonas aeruginosa* virulence. *mSphere*, 1 (2), pp.e00111-15.

Goo, K.-S. and Sim, T.-S. 2011. Designing new β-lactams: implications from their targets, resistance factors and synthesizing enzymes. *Current computer-aided drug design*, 7 (1), pp.53-80.

Goodarzi, M., Reisi-Dehkordi, N., Daryabeigi, R. and Zargham-Boroujeni, A. 2014. An epidemiologic study of burns: Standards of care and patientsâ outcomes. *Iranian Journal of Nursing and Midwifery Research*, 19 (4), pp.385-389.

Gorgani, N., Ahlbrand, S., Patterson, A. and Pourmand, N. 2009. Detection of point mutations associated with antibiotic resistance in *Pseudomonas aeruginosa*. *International Journal of Antimicrobial Agents*, 34 (5), pp.414-418.

Guzzo, F., Scognamiglio, M., Fiorentino, A., Buommino, E. and D'Abrosca, B. 2020. Plant Derived Natural Products against *Pseudomonas aeruginosa* and *Staphylococcus aureus*: Antibiofilm Activity and Molecular Mechanisms. *Molecules*, 25 (21). p.5024.

Haghi, F., Zeighami, H., Monazami, A., Toutouchi, F., Nazaralian, S. and Naderi, G. 2018. Diversity of virulence genes in multidrug resistant *Pseudomonas aeruginosa* isolated from burn wound infections. *Microbial Pathogenesis*, 115, pp.251-256.

Hainrichson, M., Yaniv, O., Cherniavsky, M., Nudelman, I., Shallom-Shezifi, D., Yaron, S. and Baasov, T. 2007. Overexpression and initial characterization of the chromosomal aminoglycoside 3′-O-phosphotransferase APH (3′)-IIb from *Pseudomonas aeruginosa*. *Antimicrobial agents and chemotherapy*, 51 (2), pp.774-776.

Hall, R.M. and Collis, C.M. 1995. Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. *Molecular microbiology*, 15 (4), pp.593-600.

Hamid, O., Robert, C., Daud, A., Hodi, F.S., Hwu, W.-J., Kefford, R., Wolchok, J.D., Hersey, P., Joseph, R.W., Weber, J.S.and Dronca,R.,2013. Safety and tumor responses with lambrolizumab (Anti–PD-1) in Melanoma. *New England Journal of Medicine*, 369 (2), pp.134-144.

Hasan, S.A., Najati, A.M. and Abass, K.S. 2019. Isolation and identification of multi-drug resistant "pseudomonas aeruginosa" from burn wound infection in kirkuk city, iraq. *EurAsian Journal of BioSciences*, 13 (2), pp.1045-1050.

Hashem, H., Hanora, A., Abdalla, S., Shawky, A. and Saad, A. 2018. *Pseudomonas aeruginosa* identifcation and MIC assessment from clinical isolates. *Records of Pharmaceutical and Biomedical Sciences*, 1 (2), pp.21-24.

Hattemer, A., Hauser, A., Diaz, M., Scheetz, M., Shah, N., Allen, J.P., Porhomayon, J. and El-Solh, A.A. 2013. Bacterial and clinical characteristics of health care-and community-acquired bloodstream infections due to *pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy*, 57 (8), pp.3969-3975.

Hawkins, C., Harper, D., Burch, D., Änggård, E. and Soothill, J. 2010. Topical treatment of *Pseudomonas aeruginosa* otitis of dogs with a bacteriophage mixture: A before/after clinical trial. *Veterinary Microbiology*, 146 (3-4), pp.309-313.

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

Hesselink, L., Heeres, M., Paraschiakos, F., Ten Berg, M., Huisman, A., Hoefer, I.E., De Groot, M.C.H., Van Solinge, W.W., Dijkgraaf, M., Hellebrekers, P., Van Wessem, K.J., 2019. A rise in neutrophil cell size precedes organ dysfunction after trauma. *Shock*, 51 (4), pp.439-446.

Hirsch, E.B. and Tam, V.H. 2010. Impact of multidrug-resistant *Pseudomonas aeruginosa* infection on patient outcomes. *Expert review of pharmacoeconomics & outcomes research*, 10 (4), pp.441-451.

Hodle, A.E., Richter, K.P. and Thompson, R.M. 2006. Infection control practices in U.S. burn units. *Journal of Burn Care and Research*, 27 (2), pp.142-151.

Hogardt, M. and Heesemann, J. 2010. Adaptation of *Pseudomonas aeruginosa* during persistence in the cystic fibrosis lung. *International Journal of Medical Microbiology*, 300 (8), pp.557-562.

Høiby, N. 2011. Recent advances in the treatment of *Pseudomonas aeruginosa* infections in cystic fibrosis. *BMC Medicine*, 9(1), pp.1-7.

Hong, D.J., Bae, I.K., Jang, I.-H., Jeong, S.H., Kang, H.-K. and Lee, K. 2015. Epidemiology and characteristics of metallo-β-lactamase-producing *Pseudomonas aeruginosa*. *Infection & chemotherapy*, 47(2), pp.81-97.

Horino, T., Chiba, A., Kawano, S., Kato, T., Sato, F., Maruyama, Y., Nakazawa, Y., Yoshikawa, K., Yoshida, M. and Hori, S. 2012. Clinical characteristics and risk factors for mortality in patients with bacteremia caused by *Pseudomonas aeruginosa*. *Internal medicine*, 51 (1), pp.59-64.

Hu, D., Wang, H., Huang, X., Jiang, Y., Qin, Y., Xiong, B., Qin, G., Sooranna, S.R. and Pinhu, L. 2016. Investigation of association between IL-8 serum levels and IL8 polymorphisms in Chinese patients with sepsis. *Gene*, 594 (1), pp.165-170.

Huang, J., Shi, Y., Zeng, G., Gu, Y., Chen, G., Shi, L., Hu, Y., Tang, B. and Zhou, J. 2016. Acyl-homoserine lactone-based quorum sensing and quorum quenching hold promise to determine the performance of biological wastewater treatments: An overview. *Chemosphere*, 157, pp.137-151.

Hussein, Z.K., Kadhim, H.S. and Hassan, J.S. 2018. Detection of New Delhi metallo-beta-lactamase-1 (blaNDM-1) in carbapenem-resistant *pseudomonas aeruginosa* isolated from clinical samples in Wasit hospitals. Iraqi JMS. 2018; 16 (3): 239-246.

Hussien, I.A., Habeb, K.A. and Jassim, K.A. 2012. Identification of *Pseudomonas aeruginosa* from burn wounds isolates by PCR using exotoxin A-specific primers. *Iraqi J. Biotech*, 11 (2), pp.282-291.

Inacio, H.S.M., Bomfim, M.R.Q., França, R.O., Farias, L.M., Carvalho, M.A.R., Serufo, J.C. and Santos, S.G. 2014. Phenotypic and genotypic diversity of multidrug-resistant *Pseudomonas aeruginosa* isolates from bloodstream infections recovered in the hospitals of Belo Horizonte, Brazil. *Chemotherapy*, 60 (1), pp.54-62.

Irazoqui, J.E., Troemel, E.R., Feinbaum, R.L., Luhachack, L.G., Cezairliyan, B.O. and Ausubel, F.M. 2010. Distinct pathogenesis and host responses during infection of C.

elegans by *P. aeruginosa* and S. aureus. *PLoS Pathogens*, 6 (7), pp.1-24.

Irfan, M., Ahmed, I., Shafee, M. and Abdul Malik Tareen, Muhammad Fazal Ur Rehman, S.A.K. 2014. Microbiological Investigation of burn patients in burn intensive units, in Quetta, Pakistan. *Journal of Infection and Molecular Biology*, 2 (4), pp.74-76.

J Wolter, D. and D Lister, P. 2013. Mechanisms of β-lactam resistance among *Pseudomonas aeruginosa*. *Current pharmaceutical design*, 19 (2), pp.209-222.

Jami Al-Ahmadi, G. and Zahmatkesh Roodsari, R. 2016. Fast and specific detection of *Pseudomonas aeruginosa* from other pseudomonas species by PCR. *Annals of burns and fire disasters*, 29 (4), pp.264-267.

Javiya, V.A., Ghatak, S.B., Patel, K.R. and Patel, J.A. 2008. Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* at a tertiary care hospital in Gujarat, India. *Indian journal of pharmacology*, 40 (5), pp.230-234.

Jawad, Z., Alshalah, A., Al-Daraghi, A. and Khaleel, A.I. 2017. Rapid detection for *lasI* and *lasR* Genes Of *Pseudomonas aeruginosa* at deference Iraqi hospitals by polymerase chain reaction (PCR) technique. *International Journal of ChemTech Research*, 10 (1), pp.409-414.

Jeschke, M.G., Gauglitz, G.G., Finnerty, C.C., Kraft, R., Mlcak, R.P. and Herndon, D.N. 2014. Survivors versus nonsurvivors postburn: Differences in inflammatory and hypermetabolic trajectories. *Annals of Surgery*, 259 (4), pp.814-823.

Jin, W.J., Xu, J.M., Xu, W.L., Gu, D.H. and Li, P.W. 2014. Diagnostic value of interleukin-8 in colorectal cancer: A case-control study and meta- Analysis. *World Journal of Gastroenterology*, 20 (43), pp.16334-16342.

Jithendra, K., Pathapati, R.M., Ponugoti, M.L., Madhavulu, B., Rao, P.S. and Sreeram, S. 2015. Time related emergence of bacteria and their antibiogram of infected burns of patients during the hospital stay. *International Journal of Current Microbiology and Applied Sciences*, 4 (1), pp.348-358.

Juan, C., Zamorano, L., Pérez, J.L., Ge, Y. and Oliver, A. 2010. Activity of a new antipseudomonal cephalosporin, CXA-101 (FR264205), against carbapenem-resistant and multidrug-resistant *Pseudomonas aeruginosa* clinical strains. *Antimicrobial agents and chemotherapy*, 54 (2), pp.846-851.

Kalia, V.C. 2013. Quorum sensing inhibitors: An overview. *Biotechnology Advances*, 31 (2), pp.224-245.

Kamat, V., Pandey, S., Paknikar, K. and Bodas, D. 2018. A facile one-step method for cell lysis and DNA extraction of waterborne pathogens using a microchip. *Biosensors and Bioelectronics*, 99, pp.62-69.

Kanagapriya, M., Pandiyaraja, S., Sucilathangam, G. and Revathy, C. 2015. Aerobic bacterial isolates in burns patients and their antibiogram. *Paripex-Indian Journal of Research*, 4 (7), pp.357-360.

Karatuna, O. and Yagci, A. 2010. Analysis of quorum sensing-dependent virulence factor production and its relationship with antimicrobial susceptibility in *Pseudomonas aeruginosa* respiratory isolates. *Clinical Microbiology and Infection*, 16 (12), pp.1770-1775.

Khajuria, A., Praharaj, A.K., Kumar, M. and Grover, N. 2013. Emergence of NDM - 1 in the clinical isolates of *Pseudomonas aeruginosa* in India. *Journal of Clinical and Diagnostic Research*, 7 (7), pp.1328-1331.

Khattab, M.A., Nour, M.S. and ElSheshtawy, N.M. 2015. Genetic identification of *Pseudomonas aeruginosa* virulence genes among different isolates. *J Microb Biochem Technol*, 7 (5), pp.274-277.

Khosravi, A.D., Motahar, M. and Abbasi Montazeri, E. 2017. The frequency of class1 and 2 integrons in *Pseudomonas aeruginosa* strains isolated from burn patients in a burn center of Ahvaz, Iran. *PloS one*, 12 (8), p.e0183061.

Kianpour, F., Havaei, S.A. and Hosseini, M.M. 2010. Evaluation of *Pseudomonas aeroginosa* isolated from cutaneous infections and determination of drug resistance pattern in patients of Alzahra hospital in Esfahan. *Journal of Isfahan Medical School*, 28 (110), pp.503-509.

Kim, M.H., Liu, W., Borjesson, D.L., Curry, F.R.E., Miller, L.S., Cheung, A.L., Liu, F.T., Isseroff, R.R. and Simon, S.I. 2008. Dynamics of neutrophil infiltration during cutaneous wound healing and infection using fluorescence imaging. *Journal of Investigative Dermatology*, 128 (7), pp.1812-1820.

Kim, S., Rahman, M. and Kim, J. 2012. Complete Genome Sequence of *Pseudomonas aeruginosa* Lytic Bacteriophage PA1O Which Resembles Temperate Bacteriophage D3112. *Journal of Virology*, 86 (6), pp.3400-3401.

Kim, H.S., Kim, J.H., Yim, H. and Kim, D. 2012. Changes in the levels of interleukins 6, 8, and 10, tumor necrosis factor alpha, and granulocyte-colony stimulating factor in Korean burn patients: Relation to burn size and postburn time. *Annals of Laboratory Medicine*, 32 (5), pp.339-344.

Kirketerp-Møller, K., Zulkowski, K. and James, G. 2011. Chronic wound colonization, infection, and biofilms. In: *Biofilm infections*. Springer.pp.11-24.

Kirkpatrick, L.A. and Brooke, C.F. 2015. *A Simple Guide to IBM SPSS Statistics*. Cengage Learning.

Klemm, E. and Dougan, G. 2016. Advances in Understanding Bacterial Pathogenesis Gained from Whole-Genome Sequencing and Phylogenetics. *Cell Host and Microbe*, 19 (5), pp.599-610.

Kombade, S.P. and Agrawal, G.N. 2014. Microbiological study of lower respiratory tract infections in ICU patients. *Int J Curr Microbiol App Sci*, 3 (8), pp.749-754.

Komolafe, O.O., James, J., Makoka, M. and Kalongeolera, L. 2003. Epidemiology and mortality of burns at the Queen Elizabeth Central Hospital Blantyre, Malawi. *The Central African journal of medicine*, 49 (11–12), pp.130-134.

Korgaonkar, A., Trivedi, U., Rumbaugh, K.P. and Whiteley, M. 2013. Community surveillance enhances *Pseudomonas aeruginosa* virulence during polymicrobial infection. *Proceedings of the National Academy of Sciences*, 110 (3), pp.1059-1064.

Köser, C.U., Ellington, M.J. and Peacock, S.J. 2014. Whole-genome sequencing to control antimicrobial resistance. *Trends in Genetics*, 30 (9), pp.401-407.

Kraft, R., Herndon, D.N., Finnerty, C.C., Cox, R.A., Song, J. and Jeschke, M.G. 2015. Predictive value of IL-8 for sepsis and severe infections after burn injury: A clinical study. *Shock*, 43 (3), pp.222-227.

Krcmery, V., Koprnova, J., Gogova, M., Grey, E. and Korcova, J. 2006. *Pseudomonas aeruginosa* bacteraemia in cancer patients. *Journal of infection*, 52 (6), pp.461-463.

Krogulski, A. 2008. Hospitals location and indoor air microbiological quality. *Roczniki Panstwowego Zakladu Higieny*, 59 (1), pp.97-102.

Kung, V.L., Ozer, E.A. and Hauser, A.R. 2010. The accessory genome of *Pseudomonas aeruginosa*. *Microbiology and Molecular Biology Reviews*, 74 (4), pp.621-641.

Kuzior, H., Fiebich, B.L., Yousif, N.M., Saliba, S.W., Ziegler, C., Nickel, K., Maier, S.J., Süß, P., Runge, K., Matysik, M., Dersch, R., 2020. Increased IL-8 concentrations in the cerebrospinal fluid of patients with unipolar depression. *Comprehensive Psychiatry*, 102, pp.4-11.

Laine, L., Perry, J.D., Lee, J., Oliver, M., James, A.L., De La Foata, C., Halimi, D., Orenga, S., Galloway, A. and Gould, F.K. 2009. A novel chromogenic medium for isolation of *Pseudomonas aeruginosa* from the sputa of cystic fibrosis patients. *Journal of Cystic Fibrosis*, 8 (2), pp.143-149.

Lam, J.S., Taylor, V.L., Islam, S.T., Hao, Y. and Kocíncová, D. 2011. Genetic and functional diversity of *Pseudomonas aeruginosa* lipopolysaccharide. *Frontiers in Microbiology*, 2, p.118.

Lambert, P.A. 2002. Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. *Journal of the royal society of medicine*, 95 (Suppl 41), p.22.

Lavery, L.A., Harkless, L.B., Felder-Johnson, K. and Mundine, S. 1994. Bacterial pathogens in infected puncture wounds in adults with diabetes. *The Journal of foot and ankle surgery: official publication of the American College of Foot and Ankle Surgeons*, 33 (1), pp.91-97.

Lee, P.Y., Costumbrado, J., Hsu, C.-Y. and Kim, Y.H. 2012. Agarose gel electrophoresis for the separation of DNA fragments. *Journal of visualized experiments : JoVE*, (62), pp.1-5.

Lewis, S.M., Bains, B.J., Bates, I. and Bain, B.J. 2001. Basic hematological techniques. *Dacie and Lewis Practical Hematology. 9th ed. London: Churchill Livingstone*, pp.19–46.

Li, H., Luo, Y.-F., Williams, B.J., Blackwell, T.S. and Xie, C.-M. 2012. Structure and function of OprD protein in *Pseudomonas aeruginosa*: from antibiotic resistance to novel therapies. *International Journal of Medical Microbiology*, 302 (2), pp.63-68.

Li, L., Yang, H., Lin, S. and Jia, S. 2010. Classification of 17 newly isolate virulent bacteriophages of *Pseudomonas aeruginosa*. *Canadian Journal of Microbiology*, 56 (11), pp.925-933.

Lila, G., Mulliqi-Osmani, G., Bajrami, R., Kurti, A., Azizi, E. and Raka, L. 2017. The prevalence and resistance patterns of *Pseudomonas aeruginosa* in a tertiary care hospital in Kosovo. *Infez Med*, 25 (1), pp.21-26.

Lima, J.L. da C., Alves, L.R., Jacomé, P.R.L. de A., Bezerra, J.P., Maciel, M.A.V., Morais, M.M.C. de, Bezerra Neto, J.P., Maciel, M.A.V. and Morais, M.M.C. de. 2018. Biofilm production by clinical isolates of *Pseudomonas aeruginosa* and structural changes in LasR protein of isolates non biofilm-producing. *Brazilian Journal of Infectious Diseases*, 22 (2), pp.129-136.

Livaditi, O., Kotanidou, A., Psarra, A., Dimopoulou, I., Sotiropoulou, C., Augustatou, K., Papasteriades, C., Armaganidis, A., Roussos, C., Orfanos, S.E. and Douzinas, E.E. 2006. Neutrophil CD64 expression and serum IL-8: Sensitive early markers of severity and outcome in sepsis. *Cytokine*, 36 (5–6), pp.283-290.

Llanes, C., Hocquet, D., Vogne, C., Benali-Baitich, D., Neuwirth, C. and Plésiat, P. 2004. Clinical strains of *Pseudomonas aeruginosa* overproducing MexAB-OprM and MexXY efflux pumps simultaneously. *Antimicrobial agents and chemotherapy*, 48 (5), pp.1797-1802.

Lloyd, E.C.O., Rodgers, B.C., Michener, M. and Williams, M.S. 2012. Outpatient burns: Prevention and care. *American Family Physician*, 85 (1), pp.25-32.

Lodise, T.P., Lomaestro, B. and Drusano, G.L. 2007. Piperacillin-tazobactam for *Pseudomonas aeruginosa* infection: Clinical implications of an extended-infusion dosing strategy. *Clinical Infectious Diseases*, 44 (3), pp.357-363.

Loman, N.J. and Pallen, M.J. 2015. Twenty years of bacterial genome sequencing. *Nature Reviews Microbiology*, 13 (12), pp.787-794.

Lopez, O.N., Cambiaso-Daniel, J., Branski, L.K., Norbury, W.B. and Herndon, D.N. 2017. Predicting and managing sepsis in burn patients: Current perspectives. *Therapeutics and Clinical Risk Management*, 13, pp.1107-1117.

López-Jácome, L.E., Garza-Ramos, G., Hernández-Durán, M., Franco-Cendejas, R., Loarca, D., Romero-Martínez, D., Nguyen, P.T.D., Maeda, T., González-Pedrajo, B., Díaz-Guerrero, M., Sánchez-Reyes, J.L., 2019. AiiM lactonase strongly reduces quorum sensing controlled virulence factors in clinical strains of *Pseudomonas aeruginosa* isolated from burned patients. *Frontiers in Microbiology*, 10, pp.1-11.

Lynch, J.P., Zhanel, G.G. and Clark, N.M. 2017. Emergence of antimicrobial resistance among *Pseudomonas aeruginosa* : implications for therapy. In: *Seminars in Respiratory and Critical Care Medicine*. Thieme Medical Publishers. Vol. 38, No. 03, pp.326-345.

Mac Aogáin, M., Kulah, C., Rijnsburger, M., Celebi, G., Savelkoul, P.H.M., O'Gara, F. and Mooij, M.J. 2012. Characterization of imipenem resistance mechanisms in *Pseudomonas aeruginosa* isolates from Turkey. *Clinical Microbiology and Infection*, 18 (7), pp.E262-265.

Macdonald, S.P.J., Stone, S.F., Neil, C.L., Van Eeden, P.E., Fatovich, D.M., Arendts, G. and Brown, S.G.A. 2014. Sustained elevation of resistin, NGAL and IL-8 are associated with severe sepsis/septic shock in the emergency department. *PLoS One*, 9 (10), p.e110678.

MacFaddin, J.F. 2000. Biochemical Tests for Identification of Medical Bacteria, Williams and Wilkins. *Philadelphia, PA*, 113.

Mahar, P., Padiglione, A.A., Cleland, H., Paul, E., Hinrichs, M. and Wasiak, J. 2010. *Pseudomonas aeruginosa* bacteraemia in burns patients: Risk factors and outcomes. *Burns*, 36 (8), pp.1228-1233.

Mahmmudi, Z., Emami, A. and Gorzin, A. 2016. Genotyping of *Pseudomonas aeruginosa* strains isolated from burn patients by RAPD-PCR molecular technique with primers 287. *International Journal of Advanced Biotechnology and Research*, 7 (2), pp.102-110.

Malešević, M., Di Lorenzo, F., Filipić, B., Stanisavljević, N., Novović, K., Senerovic, L., Polović, N., Molinaro, A., Kojić, M. and Jovčić, B. 2019. *Pseudomonas aeruginosa* quorum sensing inhibition by clinical isolate Delftia tsuruhatensis 11304: involvement of N-octadecanoylhomoserine lactones. *Scientific Reports*, 9 (1), pp.1-13.

Manandhar, S., Adhikari, S. and Rajbhandari, S. 2018. Phenotypic Assays for Detection of AmpC and MBL Producers among the Clinical Isolates of Multi Drug Resistant *Pseudomonas aeruginosa*. *Tribhuvan University Journal of Microbiology*, 4, pp.23-31.

Mardaneh, J., Abbas Poor, S. and Afrugh, P. 2013. Prevalence of Shigella species and antimicrobial resistance patterns of isolated strains from infected pediatrics in Tehran. *Int J Enteric Pathog*, 1 (1), pp.28-31.

Marra, A.R., Bar, K., Bearman, G.M.L., Wenzel, R.P. and Edmond, M.B. 2006. Systemic inflammatory response syndrome in adult patients with nosocomial bloodstream infection due to *Pseudomonas aeruginosa. Journal of Infection*, 53 (1), pp.30-35.

Masood, R.A., Wain, Z.N., Tariq, R., Ullah, M.A. and Bashir, I. 2016. Burn cases, their management and complications: A review. *International Current Pharmaceutical Journal*, 5 (12), pp.103-105.

Matsuura, H., Matsumoto, H., Osuka, A., Ogura, H., Shimizu, K., Kang, S., Tanaka, T., Ueyama, M. and Shimazu, T., 2019. Clinical importance of a cytokine network in major burns. Shock, 51(2), pp.185-193.

Maura, D. and Rahme, L.G. 2017. Pharmacological inhibition of the formation and potentiates antibiotic mediated biofilm disruption. *Antimicrob Agents Chemother*, 61 (12), pp.1-5.

McAlester, G., O'Gara, F. and Morrissey, J.P. 2008. Signal-mediated interactions between *Pseudomonas aeruginosa* and Candida albicans. *Journal of Medical Microbiology*, 57 (5), pp.563-569.

Mhada, T. V, Fredrick, F., Matee, M.I. and Massawe, A. 2012. Neonatal sepsis at Muhimbili National Hospital, Dar es Salaam, Tanzania; aetiology, antimicrobial sensitivity pattern and clinical outcome. *BMC public health*, 12 (1), pp.1-6.

Mikkelsen, H., Bond, N.J., Skindersoe, M.E., Givskov, M., Lilley, K.S. and Welch, M. 2009. Biofilms and type III secretion are not mutually exclusive in *Pseudomonas aeruginosa*. *Microbiology*, 155 (3), pp.687-698.

Mingeot-Leclercq, M.P., Glupczynski, Y. and Tulkens, P.M. 1999. Aminoglycosides: activity and resistance. *Antimicrobial Agents and Chemotherapy*, 43 (4), pp.727-737.

Miyata, S., Casey, M., Frank, D.W., Ausubel, F.M. and Drenkard, E. 2003. Use of the Galleria mellonella caterpillar as a model host to study the role of the type III secretion system in *Pseudomonas aeruginosa* pathogenesis. *Infection and Immunity*, 71 (5), pp.2404-2413.

Miyoshi, T., Yamashita, K., Arai, T., Yamamoto, K., Mizugishi, K. and Uchiyama, T. 2010. The role of endothelial interleukin-8/NADPH oxidase 1 axis in sepsis. *Immunology*,131 (3), pp.331-339.

Moghaddam, M.M. 2014. Quorum Sensing in Bacteria and a Glance on *Pseudomonas aeruginosa*. *Clinical Microbiology: Open Access*, 03 (04).

Mohammed, R.K. and Ahmad, R.A. 2017. Molecular Detection of Virulence Factors Genes in *Pseudomonas aeruginosa* isolated clinically from Different Infections Source. *Current Research in Microbiology and Biotechnology*, 5 (5), pp.1278-1284.

Mohanty, S., Baliyarsingh, B. and Kumar Nayak, S. 2021. Antimicrobial Resistance in *Pseudomonas aeruginosa* : A Concise review . *Antimicrobial Resistance - A One Health Perspective*.

Mokhtari, A. and Amini, K. 2019. Genotyping of *Pseudomonas Aeruginosa* strains as a multidrug resistant (MDR) bacterium and evaluating the prevalence of esbls and some virulence factors encoding genes by PFGE and ERIC-PCR methods. *Iranian Journal of Pharmaceutical Research*, 18 (3), pp.1580-1594.

Momin, M.H.F.A., Bean, D.C., Hendriksen, R.S., Haenni, M., Phee, L.M. and Wareham, D.W. 2017. CHROMagar COL-APSE: a selective bacterial culture medium for the isolation and differentiation of colistin-resistant Gram-negative pathogens. *Journal of medical microbiology*, 66 (11), pp.1554-1561.

Mooij, M.J., Drenkard, E., Llamas, M.A., Vandenbroucke-Grauls, C.M.J.E., Savelkoul, P.H.M., Ausubel, F.M. and Bitter, W. 2007. Characterization of the integrated filamentous phage Pf5 and its involvement in small-colony formation. *Microbiology*, 153 (Pt 6), p.1790.

Morrison, A.J. and Wenzel, R.P. 2015. Epidemiology of infections due to *Pseudomonas aeruginosa*. *Reviews of infectious diseases*, *6*(Supplement-3), pp.S627-S642.

Moser, B. and Loetscher, P. 2001. Lymphocyte traffic control by chemokines. *Nature Immunology*, 2 (2), pp.123-128.

Moyá, B., Zamorano, L., Juan, C., Ge, Y. and Oliver, A. 2010. Affinity of the new cephalosporin CXA-101 to penicillin-binding proteins of *Pseudomonas aeruginosa*. *Antimicrobial agents and chemotherapy*, 54 (9), pp.3933-3937.

Moya, B., Dötsch, A., Juan, C., Blázquez, J., Zamorano, L., Haussler, S. and Oliver, A. 2009. β-Lactam resistance response triggered by inactivation of a nonessential penicillin-binding protein. *PLoS pathogens*, 5 (3), p.e1000353.

Mukerji, G., Chamania, S., Patidar, G.P. and Gupta, S. 2001. Epidemiology of paediatric burns in Indore, India. *Burns*, 27 (1), pp.33-38.

Mulcahy, L.R., Burns, J.L., Lory, S. and Lewis, K. 2010. Emergence of *Pseudomonas aeruginosa* strains producing high levels of persister cells in patients with cystic fibrosis. *Journal of Bacteriology*, 192 (23), pp.6191-6199.

Mulet, M., Lalucat, J. and García-Valdés, E. 2010. DNA sequence-based analysis of the Pseudomonas species. *Environmental microbiology*, 12 (6), pp.1513-1530.

Munita, J.M. and Arias, C.A. 2016. Mechanisms of antibiotic resistance. *Virulence Mechanisms of Bacterial Pathogens*, pp.481-511.

Nazzaro, F., Fratianni, F. and Coppola, R. 2013. Quorum sensing and phytochemicals. *International Journal of Molecular Sciences*, 14 (6), pp.12607-12619.

Neamah, A.A. 2017. Molecular detection of virulence factor genes in *pseudomonas aeruginosa* isolated from human and animals in Diwaniya province. *Kufa Journal For Veterinary Medical Sciences*, 8 (1), pp.218-230.

Nikaido, H. 1994. Prevention of drug access to bacterial targets: Permeability barriers and active efflux. *Science*, 264 (5157), pp.382-388.

Nikbin, V.S., Aslani, M.M., Sharafi, Z., Hashemipour, M., Shahcheraghi, F. and Ebrahimipour, G.H. 2012. Molecular identification and detection of virulence genes among *Pseudomonas aeruginosa* isolated from different infectious origins. *Iranian journal of microbiology*, 4 (3), p.118.

Nikokar, I., Tishayar, A., Flakiyan, Z., Alijani, K., Rehana-Banisaeed, S., Hossinpour, M., Amir-Alvaei, S. and Araghian, A. 2013. Antibiotic resistance and frequency of class 1 integrons among *Pseudomonas aeruginosa*, isolated from burn patients in Guilan, Iran. *Iranian journal of microbiology*, 5 (1), p.36.

Ochsner, U.A., Wilderman, P.J., Vasil, A.I. and Vasil, M.L. 2002. GeneChip® expression analysis of the iron starvation response in *Pseudomonas aeruginosa*: Identification of novel pyoverdine biosynthesis genes. *Molecular Microbiology*, 45 (5), pp.1277-1287.

Odumosu, B.T., Adeniyi, B.A. and Chandra, R. 2013. Analysis of integrons and associated gene cassettes in clinical isolates of multidrug resistant *Pseudomonas aeruginosa* from Southwest Nigeria. *Annals of clinical microbiology and antimicrobials*, 12 (1), pp.1-7.

Oncul, O., Ulkur, E., Acar, A., Turhan, V., Yeniz, E., Karacaer, Z. and Yildiz, F. 2009. Prospective analysis of nosocomial infections in a burn care unit, Turkey. *Indian Journal of Medical Research*, 130 (6), pp.758-764.

Osayande, J.O. 2010. Easy identification of difficult-to-type *Pseudomonas aeruginosa* clinical and environmental isolates. *Internet Journal of Microbiology*, 7 (2).

Otte, C., Gold, S.M., Penninx, B.W., Pariante, C.M., Etkin, A., Fava, M., Mohr, D.C. and Schatzberg, A.F. 2016. Major depressive disorder. *Nature reviews Disease primers*, 2 (1), pp.1-20.

Otter, J.A., Vickery, K., Walker, J.T., deLancey Pulcini, E., Stoodley, P., Goldenberg, S.D., Salkeld, J.A.G., Chewins, J., Yezli, S. and Edgeworth, J.D. 2015. Surface-attached cells, biofilms and biocide susceptibility: Implications for hospital cleaning anddisinfection. *Journal of Hospital Infection*, 89 (1), pp.16-27.

Özen, A.I. and Ussery, D.W. 2012. Defining the Pseudomonas genus: where do we draw the line with Azotobacter? *Microbial Ecology*, 63 (2), pp.239-248.

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

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

Panjeshahin, M.R., Lari, A.R., Talei, A.R., Shamsnia, J. and Alaghehbandan, R. 2001. Epidemiology and mortality of burns in the South West of Iran. *Burns*,27 (3), pp.219-226.

Parsa, P., Amirmozafari, N., Nowruzi, B. and Bahar, M.A. 2020. Molecular characterization of polymorphisms among *Pseudomonas aeruginosa* strains isolated from burn patients' wounds. *Heliyon*, 6 (12), p.e05041.

Paterson, D.L. and Bonomo, R.A. 2005. Clinical update extended-spectrum beta-lactamases : a clinical update. *Clinical Microbiology Reviews*, 18 (4), pp.657-686.

Peck, M.D. 2011. Epidemiology of burns throughout the world. Part I: Distribution and risk factors. *Burns*, 37 (7), pp.1087-1100.

Peck, M.D., Kruger, G.E., van der Merwe, A.E., Godakumbura, W. and Ahuja, R.B. 2008. Burns and fires from non-electric domestic appliances in low and middle income countries. Part I. The scope of the problem. *Burns*, 34 (3), pp.303-311.

Peix, A., Ramírez-Bahena, M.H. and Velázquez, E. 2009. Historical evolution and current status of the taxonomy of genus Pseudomonas. Infection, *Genetics and Evolution*, 9 (6), pp.1132-1147.

Peranantham, Manigandan, G. and Shanmugam, K., 2014. Forensic approach to a case of death due to burn injury: a case report. *International Journal of Research in Medical Sciences*, 2 (3), p.1214-6.

Pereima, M.J.L., Leal, M., Capella, M.R., Goldberg, P., Quaresma, E.R., Araújo, E.J. and Souza, J.A. 2001. Análise de 573 crianças com queimaduras internadas no Hospital Infantil Joana de Gusmão. *Rev Bras Queimaduras*, 1 (1), pp.41-48.

Perez, L.R.R., Costa, M.C.N., Freitas, A.L.P. de and Barth, A.L. 2011. Evaluation of biofilm production by *Pseudomonas aeruginosa* isolates recovered from cystic fibrosis and non-cystic fibrosis patients. *Brazilian Journal of Microbiology*, 42, pp.476-479.

Perez, L.R.R., Machado, A.B.M.P. and Barth, A.L. 2013. The presence of quorum-sensing genes in Pseudomonas isolates infecting cystic fibrosis and non-cystic fibrosis patients. *Current microbiology*, 66 (4), pp.418-420.

Pérez-Velázquez, J., Gölgeli, M. and García-Contreras, R. 2016. Mathematical modelling of bacterial quorum sensing: a review. *Bulletin of Mathematical Biology*, 78 (8), pp.1585-1639.

Perletti, G., Magri, V., Wagenlehner, F.M.E. and Naber, K.G. 2010. CXA-101. *Drugs Fut*, 35 (12), pp.977-986.

Pestrak, M.J., Baker, P., Dellos-Nolan, S., Hill, P.J., Passos da Silva, D., Silver, H., Lacdao, I., Raju, D., Parsek, M.R., Wozniak, D.J. and Lynne Howell, P. 2019. Treatment with the *pseudomonas aeruginosa* glycoside hydrolase PslG combats wound infection by improving antibiotic efficacy and host innate immune activity. *Antimicrobial Agents and Chemotherapy*, 63 (6). pp.e00234-19.

Poole, K. 2004. Resistance to β-lactam antibiotics. *Cellular and Molecular Life Sciences CMLS*, 61 (17), pp.2200-2223.

Poole, K. 2005. Aminoglycoside resistance in *Pseudomonas aeruginosa*. *Antimicrobial agents and Chemotherapy*, 49 (2), pp.479-487.

Poonsuk, K., Tribuddharat, C. and Chuanchuen, R. 2014. Simultaneous overexpression of multidrug efflux pumps in *Pseudomonas aeruginosa* non-cystic fibrosis clinical isolates. *Canadian journal of microbiology*, 60 (7), pp.437-443.

Priebe, G.P. and Goldberg, J.B. 2014. Vaccines for pseudomonas aeruginosa: A long and winding road. *Expert Review of Vaccines*, 13 (4), pp.507-519.

Procop, G.W., Church, D.L., Hall, G.S. and Janda, W.M. 2020. *Koneman's color atlas and textbook of diagnostic microbiology*. Jones & Bartlett Publishers.

Puma, C., Danik, M., Quirion, R., Ramon, F. and Williams, S. 2001. The chemokine interleukin-8 acutely reduces Ca2+ currents in identified cholinergic septal neurons expressing CXCR1 and CXCR2 receptor mRNAs. *Journal of neurochemistry*, 78 (5), pp.960-971.

Rafla, K. and Tredget, E.E. 2011. Infection control in the burn unit. *Burns*, 37 (1), pp.5–15.

Rajput, A., Singh, K.P., Kumar, V., Sexena, R. and Singh, R.K. 2008. Antibacterial resistance pattern of aerobic bacteria isolates from burn patients in tertiary care hospital. *Biomedical Research*, 19 (1), pp.5-8.

Randall, D.R. and Randall, D.R. 2009. Molecular Biology Laboratory Manual. *Spring*.

Ranjbar, M., Behrouz, B., Norouzi, F. and Mousavi Gargari, S.L. 2019. Anti-PcrV IgY antibodies protect against *Pseudomonas aeruginosa* infection in both acute pneumonia and burn wound models. *Molecular Immunology*, 116, pp.98-105.

Ranjbar, R., Owlia, P., Saderi, H., Mansouri, S., Jonaidi-Jafari, N., Izadi, M., Farshad, S. and Arjomandzadegan, M. 2011. Characterization of *Pseudomonas aeruginosa* strains isolated from burned patients hospitalized in a major burn center in Tehran, Iran. *Acta Med Iran*, 49 (10), pp.675-679.

Rawat, D. and Nair, D. 2010. Extended-spectrum ß-lactamases in gram negative bacteria. *Journal of Global Infectious Diseases*, 2 (3), p.263.

Reading, N.C. and Sperandio, V. 2006. Quorum sensing: The many languages of bacteria. *FEMS Microbiology Letters*, 254 (1), pp.1-11.

Rehman, A., Patrick, W.M. and Lamont, I.L. 2019. Mechanisms of ciprofloxacin resistance in *Pseudomonas aeruginosa*: new approaches to an old problem. *Journal of Medical Microbiology*, 68 (1), pp.1-10.

Remans, K., Vercammen, K., Bodilis, J. and Cornelis, P. 2010. Genome-wide analysis and literature-based survey of lipoproteins in *Pseudomonas aeruginosa*. *Microbiology*,156 (9), pp.2597-2607.

Remick, D.G. 2005. Interleukin-8. *Critical care medicine*, 33 (12), pp.S466-S467.

Ritter, M., Schratzberger, P., Rossmann, H., Wöll, E., Seiler, K., Seidler, U., Reinisch, N., Kähler, C.M., Zwierzina, H., Lang, H.J., Lang, F.,1998. Effect of inhibitors of Na+/H+-exchange and gastric H+/K+ ATPase on cell volume, intracellular pH and migration of human polymorphonuclear leucocytes. *British Journal of Pharmacology*, 124 (4), pp.627-638.

Robinson, E.R., Walker, T.M. and Pallen, M.J. 2013. Genomics and outbreak investigation: From sequence to consequence. *Genome Medicine*, 5 (4), pp.1-9.

Roetzer, A., Diel, R., Kohl, T.A., Rückert, C., Nübel, U., Blom, J., Wirth, T., Jaenicke, S., Schuback, S. and Rüsch-Gerdes, S. 2013. Whole genome sequencing versus traditional genotyping for investigation of a Mycobacterium tuberculosis outbreak: a longitudinal molecular epidemiological study. *PLoS medicine*, 10 (2), p.e1001387.

Rossolini, G.M. and Mantengoli, E. 2005. Treatment and control of severe infections caused by multiresistant *Pseudomonas aeruginosa*. *Clinical Microbiology and infection*, 11, pp.17-32.

Rutala, W.A. and Weber, D.J. 2001. New disinfection and sterilization methods. In: *Emerging Infectious Diseases*. pp.348-353.

Sabharwal, N., Dhall, S., Chhibber, S. and Harjai, K. 2014. Molecular detection of virulence genes as markers in *Pseudomonas aeruginosa* isolated from urinary tract infections. *International journal of molecular epidemiology and genetics*, 5 (3), p.125.

Salehi, Z., Amini, K. and Kheirkhah, B. 2017. Molecular identification of quorum sensing genes in clinical strains of *Pseudomonas aeruginosa* and antibiotic resistance profile. *Journal of Babol University of Medical Sciences*, 19 (4), pp.46-53.

Sambrook, J. and Russell, D.W. 2001. Molecular Cloning-Sambrook & Russel-Vol. 1, 2, 3. *Cold Springs Harbour Laboratory Press*.

Sambrook, J. and Russell, D.W. 2006. Agarose gel electrophoresis. *Cold Spring Harbor Protocols*, 2006 (1), p.pdb-prot4020.

Samimi, R., Fatemi, M.J. and Soltani, M. 2011.The epidemiological assessment of burn injuries in children admitted to Mottahari Hospital, Tehran, 2009-2010. *Iranian Journal of Surgery*.

Samuel, J.C., Campbell, E.L.P., Mjuweni, S., Muyco, A.P., Cairns, B.A. and Charles, A.G. 2011. The epidemiology, management, outcomes and areas for improvement of burn care in Central Malawi: An observational study. *Journal of International Medical Research*, 39 (3), pp.873-879.

Samuel, S.., Kayode, O.., Musa, O.., Nwigwe, G.., Aboderin, A.., Salami, T.A.. and Taiwo, S.. 2010. Nosocomial infections and the challenges of control in developing countries. *African Journal of Clinical and Experimental Microbiology*, 11 (2), pp.102-110.

Santucci, S.G., Gobara, S., Santos, C.R., Fontana, C. and Levin, A.S. 2003. Infections in a burn intensive care unit: experience of seven years. *Journal of Hospital Infection*, 53 (1), pp.6-13.

Sauvage, E., Kerff, F., Terrak, M., Ayala, J.A. and Charlier, P. 2008. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. *FEMS microbiology reviews*, 32 (2), pp.234-258.

Schaber, J.A., Carty, N.L., McDonald, N.A., Graham, E.D., Cheluvappa, R., Griswold, J.A. and Hamood, A.N. 2004. Analysis of quorum sensing-deficient clinical isolates of *Pseudomonas aeruginosa*. *Journal of Medical Microbiology*, 53 (9), pp.841-853.

Schaible, B., Crifo, B., Schaffer, K. and Taylor, C.T. 2020. The putative bacterial oxygen sensor Pseudomonas prolyl hydroxylase (PPHD) suppresses antibiotic resistance and pathogenicity in *Pseudomonas aeruginosa*. *Journal of Biological Chemistry*, 295 (5), pp.1195-1201.

Schechner, V., Nobre, V., Kaye, K.S., Leshno, M., Giladi, M., Rohner, P., Harbarth, S., Anderson, D.J., Karchmer, A.W., Schwaber, M.J. and Carmeli, Y. 2009. Gram-negative bacteremia upon hospital admission: when should *Pseudomonas aeruginosa* be suspected? *Clinical Infectious Diseases*, 48 (5), pp.580-586.

Schraufstatter, I.U., Chung, J. and Burger, M. 2001. IL-8 activates endothelial cell CXCR1 and CXCR2 through Rho and Rac signaling pathways. *American Journal of Physiology - Lung Cellular and Molecular Physiology*, 280 (6 24-6), pp.L1094-L1103.

Selim, S., El Kholy, I., Hagagy, N., El Alfay, S. and Abdel Aziz, M. 2015. Rapid identification of *Pseudomonas aeruginosa* by pulsed-field gel electrophoresis. *Biotechnology and Biotechnological Equipment*, 29 (1), pp.152-156.

Semple, B.D., Kossmann, T. and Morganti-Kossmann, M.C. 2010. Role of chemokines in CNS health and pathology: a focus on the CCL2/CCR2 and CXCL8/CXCR2 networks. *Journal of Cerebral Blood Flow & Metabolism*, 30 (3), pp.459-473.

Shahandashti, E.F., Molana, Z., Asgharpour, F., Mojtahedi, A. and Rajabnia, R. 2012. Molecular detection of Integron genes and pattern of antibiotic resistance in *Pseudomonas aeruginosa* strains isolated from intensive care unit, Shahid Beheshti Hospital, North of Iran. *International journal of molecular and cellular medicine*, 1 (4), p.209.

Shank, E.S., Sheridan, R.L., Coté, C.J. and Martyn, J.A.J. 2009. Burn injuries. *A Practice of Anesthesia for Infants and Children*, 302 (16), pp.715-733.

Sharma, G., Rao, S., Bansal, A., Dang, S., Gupta, S. and Gabrani, R. 2014. *Pseudomonas aeruginosa* biofilm: Potential therapeutic targets. *Biologicals*, 42 (1), pp.1-7.

Sharmeen, R., Hossain, MN., Rahman, M.M., Foysal, M.J. and Miah, M.F. 2012. In-vitro antibacterial activity of herbal aqueous extract against multi-drug resistant *Klebsiella* sp. isolated from human clinical samples. *International Current Pharmaceutical Journal*, 1 (6), pp.133-137.

Shehab, Z.H., Ahmed, S.T. and Abdallah, N.M. 2020. Genetic variation of pilB gene in *Pseudomonas aeruginosa* isolated from Iraqi patients with burn infections. *Annals of Tropical Medicine and Public Health*, 23 (16), pp.231-615.

Shenoy, S., Baliga, S., Saldanha, D.R.M. and Prashanth, H. V. 2002. Antibiotic sensitivity patterns of *Pseudomonas aeruginosa* strains isolated from various clinical specimens. *Indian Journal of Medical Sciences*, 56 (9), pp.427-430.

Sherman, N.E., Stefansson, B., Fox, J.W. and Goldberg, J.B. 2001. Pseudomonas aeruginosa and a proteomic approach to bacterial pathogenesis. *Disease Markers*, 17 (4), pp.285-293.

Shewatatek, G., Gizachew, T., Molalegne, B. and Terefe, G. 2014. Drug sensitivity of *Pseudomonas aeruginosa* from wound infections in Jimma University Specialized Hospital, Ethiopia. *J. Med. Med. Sci. Res*, 3 (2), pp.13-18.

Shrestha, S., Amatya, R. and Adhikari, R.P. 2015. Prevalence and antibiogram of *Pseudomonas aeruginosa* isolated from clinical specimens in a Teaching Hospital, Kathmandu. *Nepal Med Coll J*, 17 (3–4), pp.132-135.

Silva, A., Silva, V., Igrejas, G. and Poeta, P. 2019. Carbapenems and *Pseudomonas aeruginosa*: mechanisms and epidemiology. In: *Antibiotics and Antimicrobial Resistance Genes in the Environment:* volume 1.pp.253-268.

Simchowitz, L. and Cragoe, E.J. 1986. Regulation of human neutrophil chemotaxis by intracellular pH. *Journal of Biological Chemistry*, 261 (14), pp.6492-6500.

Singh, N.P., Rani, M., Gupta, K., Sagar, T. and Kaur, I.R. 2017. Changing trends in antimicrobial susceptibility pattern of bacterial isolates in a burn unit. *Burns*, 43 (5), pp.1083-1087.

Siviero Do Vale, E.C. 2005. Inicial management of burns: Approach by dermatologists. *Anais Brasileiros de Dermatologia*, 80 (1), pp.9-19.

Sivri, N., Jones, M. and Allen, M.J. 2014. *Pseudomonas aeruginosa* Isolated From the Marine Environments in the Istanbul Coastal Area (Turkey). *Fresenius Environmental Bulletin*, 23 (12B), p.1.

Sloniecka, M., Roux, S.L.E., Zhou, Q. and Danielson, P. 2016. Substance p enhances keratocyte migration and neutrophil recruitment through interleukin-8. *Molecular Pharmacology*, 89 (2), pp.215-225.

Slovis, C. 2011. *EMS: A Practical Global Guidebook. JEMS: Journal of Emergency Medical Services*, PMPH-USA.

Smith, R.S. and Iglewski, B.H. 2003. *Pseudomonas aeruginosa* quorum sensing as a potential antimicrobial target. *The Journal of clinical investigation*, 112 (10), pp.1460-1465.

Snyder, L.A., Loman, N.J., Faraj, L.A., Levi, K., Weinstock, G., Boswell, T.C., Pallen, M.J. and Ala'Aldeen, D.A. 2013. Epidemiological investigation of *pseudomonas aeruginosa* isolates from a six-year-long hospital outbreak using high-throughput whole genome sequencing. *Eurosurveillance*, 18 (42), p.20611.

Soini, H. and Musser, J.M. 2001. Molecular diagnosis of *mycobacteria*. *Clinical Chemistry*, 47(5), pp.809-814.

Somily, A.M., Absar, M.M., Arshad, M.Z., Al Aska, A.I., Shakoor, Z.A., Fatani, A.J., Siddiqui, Y.M. and Murray, T.S. 2012. Antimicrobial susceptibility patterns of multidrugresistant *pseudomonas aeruginosa* and acinetobacter baumannii against carbapenems, colistin, and tigecycline. *Saudi Medical Journal*, 33 (7), pp.750-755.

Sousa, D., Ceniceros, A., Galeiras, R., Pértega-Díaz, S., Gutiérrez-Urbón, J.M., Rodríguez-Mayo, M., López-Suso, E., Mourelo-Fariña, M. and Llinares, P. 2018. Microbiology in burns patients with blood stream infections: trends over time and during the course of hospitalization. *Infectious Diseases*, 50 (4), pp.289-296.

Sprague, A.H. and Khalil, R.A. 2009. Inflammatory cytokines in vascular dysfunction and vascular disease. *Biochemical Pharmacology*, 78 (6), pp.539-552.

Stampolidis, N., Castana, O., Nikiteas, N., Vlasis, K., Koupidis, S.A., Grammatikopoulos, I.A., Mantzari, E., Pallantzas, A., Kourakos, P. and Papadopoulos, O. 2012. Quality of life in burn patients in greece. *Annals of Burns and Fire Disasters*, 25 (4), pp.192-195.

Starkey, M., Lepine, F., Maura, D., Bandyopadhaya, A., Lesic, B., He, J., Kitao, T., Righi, V., Milot, S., Tzika, A. and Rahme, L. 2014. Identification of anti-virulence compounds that disrupt quorum-sensing regulated acute and persistent pathogenicity. *PLoS Pathogens*, 10 (8), p.e1004321.

Steinstraesser, L., Oezdogan, Y., Wang, S.C. and Steinau, H.U. 2004. Host defense peptides in burns. *Burns*, 30 (7), pp.619-627.

Streeter, K. and Katouli, M. 2016. *Pseudomonas aeruginosa*: A review of their pathogenesis and prevalence in clinical settings and the environment. *Infection, Epidemiology and Medicine*, 2 (1), pp.25-32.

Stuart, M.J. and Baune, B.T. 2014. Chemokines and chemokine receptors in mood disorders, schizophrenia, and cognitive impairment: A systematic review of biomarker studies. *Neuroscience and Biobehavioral Reviews*, 42, pp.93-115.

Stuart, M.J., Singhal, G. and Baune, B.T. 2015. Systematic review of the neurobiological relevance of chemokines to psychiatric disorders. *Frontiers in cellular neuroscience*, 9, p.357.

Sun, S.S., Chen, Y., Yang, J., Tian, T.F., Deng, H.X., Li, W.H., Du, H. and Alici, G. 2014. The development of an adaptive tuned magnetorheological elastomer absorber working in squeeze mode. *Smart Materials and Structures*, 23 (7), p.75009.

Tannous, E., Lipman, S., Tonna, A., Hector, E., Hussein, Z., Stein, M. and Reisfeld, S. 2020. Time above the MIC of Piperacillin-tazobactam as a predictor of outcome in *pseudomonas aeruginosa* bacteremia. *Antimicrobial Agents and Chemotherapy*, 64 (8), pp.e02571-19.

Tayh, G. a. 2013. Risk factors and antimicrobial resistance of pathogens isolated from burn units at local hospitals in Gaza Strip , Palestine. *Burns*, 8, pp.1612-1618.

Tayh, G.A., AL Laham, N.A., Elmanama, A.A. and Slama, K. Ben. 2016. Occurrence and antimicrobial susceptibility pattern of ESBL among Gram-negative bacteria isolated from burn unit of Al Shifa hospital in Gaza, Palestine. *The International Arabic Journal of Antimicrobial Agents*, 5 (3).

Tekin, R., Yolbas, I., Dal, T., Okur, M.H. and Selçuk, C.T. 2013. The evaluation of patients with burns during fifteen years period. *Clinica Terapeutica*, 164 (5), pp.385-389.

Todar K. 2004. Todar's Online Textbook of Bacteriology. *Pseudomonas aeruginosa*, pp.396-404.

Toussaint, J. and Singer, A.J. 2014. The evaluation and management of thermal injuries: 2014 update. *Clinical and Experimental Emergency Medicine*, 1 (1), pp.8-18.

Tümmler, B. 2019. Emerging therapies against infections with *pseudomonas aeruginosa*. *F1000Research*, 8, pp.1-14.

Turner, K.H., Everett, J., Trivedi, U., Rumbaugh, K.P. and Whiteley, M. 2014. Requirements for *Pseudomonas aeruginosa* Acute burn and chronic surgical wound infection. *PLoS Genetics*, 10 (7), p.e1004518.

Uboncholakate, K., Sikong, L. and Saito, F. 2012. Photocatalytic disinfection of *P.aeruginosa* bacterial Ag-doped TiO 2 film. *Procedia Engineering*, 32, pp.656-662.

Vadukul, K. 2012. Burn Injuries Require Quick Identification, Treatment & Transport. *Journal of Emergency Medical Services*, 37, p.12.

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

Venturi, V. 2006. Regulation of quorum sensing in Pseudomonas. *FEMS microbiology reviews*, 30 (2), pp.274-291.

Verhoeff, M., Van Der Veen, E.L., Rovers, M.M., Sanders, E.A.M. and Schilder, A.G.M. 2006. Chronic suppurative otitis media: A review. *International Journal of Pediatric Otorhinolaryngology*, 70 (1), pp.1-12.

Vincent, C., Taylor-Adams, S., Chapman, E.J., Hewett, D., Prior, S., Strange, P. and Tizzard, A. 2000. How to investigate and analyse clinical incidents: clinical risk unit and association of litigation and risk management protocol. *British Medical Journal*, 320 (7237), pp.777-781.

Vitkauskienė, A., Skrodenienė, E., Dambrauskienė, A., Macas, A. and Sakalauskas, R. 2010. *Pseudomonas aeruginosa* bacteremia: resistance to antibiotics, risk factors, and patient mortality. *Medicina*, 46 (7), p.490.

Von Bodman, S.B., Willey, J.M. and Diggle, S.P. 2008. Cell-cell communication in bacteria: United we stand. In: *Journal of Bacteriology*. pp.4377-4391.

Wan Yusoff, W.S.Y., Abdullah, M., Sekawi, Z., Amran, F., Yuhana, M.Y., Mohd Taib, N., Md. Shah, A. and Amin Nordin, S. 2020. Elevated levels of IL-8 in fatal leptospirosis. *Pathogens and Global Health*, 114 (2), pp.99-103.

Wang, C., McPherson, J.R., Zhang, L.H., Rozen, S. and Sabapathy, K. 2016. Transcription-associated mutation of *lasR* in *Pseudomonas aeruginosa*. *DNA Repair*, 46, pp.9-19.

Waugh, D.J.J. and Wilson, C. 2008. The interleukin-8 pathway in cancer. *Clinical cancer research*, 14 (21), pp.6735-6741.

Weber, D.J., Rutala, W.A., Miller, M.B., Huslage, K. and Sickbert-Bennett, E. 2010. Role of hospital surfaces in the transmission of emerging health care-associated pathogens: norovirus, *Clostridium* difficile, and *Acinetobacter* species. *American Journal of Infection Control*, 38 (5), pp.S25-S33.

Wei, Q. and Ma, L.Z. 2013. Biofilm matrix and its regulation in *Pseudomonas aeruginosa*. *International Journal of Molecular Sciences*, 14 (10), pp.20983-21005.

Welte, W., Nestel, U., Wacker, T. and Diederichs, K. 1995. Structure and function of the porin channel. *Kidney International*, 48 (4), pp.930-940.

Wessel, A.K., Liew, J., Kwon, T., Marcotte, E.M. and Whiteley, M. 2013. Role of *Pseudomonas aeruginosa* peptidoglycan-associated outer membrane proteins in vesicle formation. *Journal of Bacteriology*, 195 (2), pp.213-219.

Williams, B.J., Dehnbostel, J. and Blackwell, T.S. 2010. *Pseudomonas aeruginosa*: host defence in lung diseases. *Respirology*, 15 (7), pp.1037–1056.

Williams, G., Borkakoti, N., Bottomley, G.A., Cowan, I., Fallowfield, A.G., Jones, P.S., Kirtland, S.J., Price, G.J. and Price, L. 1996. Mutagenesis Studies of Interleukin-8. *Journal of Biological Chemistry*, 271 (16), pp.9579-9586.

Wright, G.D. 2005. Bacterial resistance to antibiotics: Enzymatic degradation and modification. *Advanced Drug Delivery Reviews*, 57 (10), pp.1451-1470.

Wu, S.J., Chan, A. and Kado, C.I. 2004. Detection of PCR amplicons from bacterial pathogens using microsphere agglutination. *Journal of Microbiological Methods*, 56 (3), pp.395-400.

Yang, Y., Rasmussen, B.A. and Shlaes, D.M. 1999. Class A β-lactamases-enzyme-inhibitor interactions and resistance. *Pharmacology & therapeutics*, 83 (2), pp.141-151.

Yayan, J., Ghebremedhin, B. and Rasche, K. 2015. Antibiotic resistance of *Pseudomonas aeruginosa* in pneumonia at a single university hospital center in Germany over a 10-year period. *Plos one*, 10 (10), p.e0139836.

Yeh, F.L., Lin, W.L., Shen, H.D. and Fang, R.H. 1997. Changes in levels of serum IL-8 in burned patients. *Burns*, 23 (7–8), pp.555-559.

Yousefi-Mashouf, R. and Hashemi, S.H. 2006. The epidemiology of burn wound infections in patients hospitalized in burn center of Hamedan, Western Iran. *Journal of Medical Sciences*, 6 (3), pp.426-431.

Zapun, A., Contreras-Martel, C. and Vernet, T. 2008. Penicillin-binding proteins and β-lactam resistance. *FEMS microbiology reviews*, 32 (2), pp.361-385.

Zavascki, A.P., Goldani, L.Z., Li, J. and Nation, R.L. 2007. Polymyxin B for the treatment of multidrug-resistant pathogens: a critical review. *Journal of antimicrobial chemotherapy*, 60 (6), pp.1206-1215.

Zeb, A., Ullah, I., Rehman, H., Rehman, M., Jadoon, M.A., Ullah, I., Alam, I., Ullah, R., Iqbal, Z., Hameed, M.F. and Haya, A. 2017. Antibiotic susceptibility pattrens of *Pseudomonas aeruginosa* in tertiary care hospital. *Entomology and Zoology Studies*, 5 (1), pp.437-439.

Appendix

Appendix (1).

The result antibiotic susceptibility test *P. aeruginosa* isolated from burned patients by using VITEIK2.

Isolate	Antimicrobial																			
	TZP		CZ		CAZ		FEP		IPM		AN		GM		CIP		LEV		TGC	
	INP	MIC	INP	MIC	INP	MIC	INP	MIC	INP	MIC	INP	MIC	INP	MIC	INP	MIC	INP	MIC	INP	MIC
S1	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S2	S	<=4	*R	<=4	S	8	S	4	S	<=0.25	I	32	S	4	S	0.5	S	1	*R	<=0.5
S3	S	<=4	R	32	S	4	S	4	S	0.5	S	<=2	R	>=16	S	<=0.25	S	<=0.12	*R	<=0.5
S4	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S5	S	<=4	R	>=64	I	16	S	2	S	0.5	S	<=2	R	>=16	I	2	S	1	S	<=0.5
S6	S	16	R	>=64	S	4	I	16	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S7	S	16	R	>=64	R	>=64	R	>=64	R	>=16	S	16	I	8	R	>=4	R	>=8	*R	4
S8	S	8	R	>=64	S	2	S	<=1	S	2	S	<=2	S	2	S	<=0.25	S	0.5	R	>=8
S9	S	32	R	>=64	S	4	I	16	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S10	S	64	R	>=64	*I	4*	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S11	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S12	S	32	R	>=64	S	4	I	16	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S13	S	32	R	>=64	R	>=64	R	32	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S14	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S15	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S16	S	16	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	S	4	R	>=4	R	>=8	R	>=8
S17	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S18	S	16	R	>=64	R	>=64	R	>=64		>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S19	S	32	R	>= 64	I	16	S	8	S	<=0.25	S	16	S	<=1	R	>=4	S	1	S	2
S20	S	16	R	>=64	R	>=64	S	4	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S21	S	<=4	*R	8	R	32	*I	4*	S	<=0.25	R	>=64	R	>=16	R	>=4	R	>=8	*R	<=0.5
S22	S	16	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	S	4	R	>=4	R	>=8	R	>=8
S23	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S24	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S25	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S26	S	32	R	>=64	S	4	I	16	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S27	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S28	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S29	S	32	R	>=64	S	4	R	32	R	>=16	R	>=64	R	>=16	R	>=4	S	1	*R	<=0.5

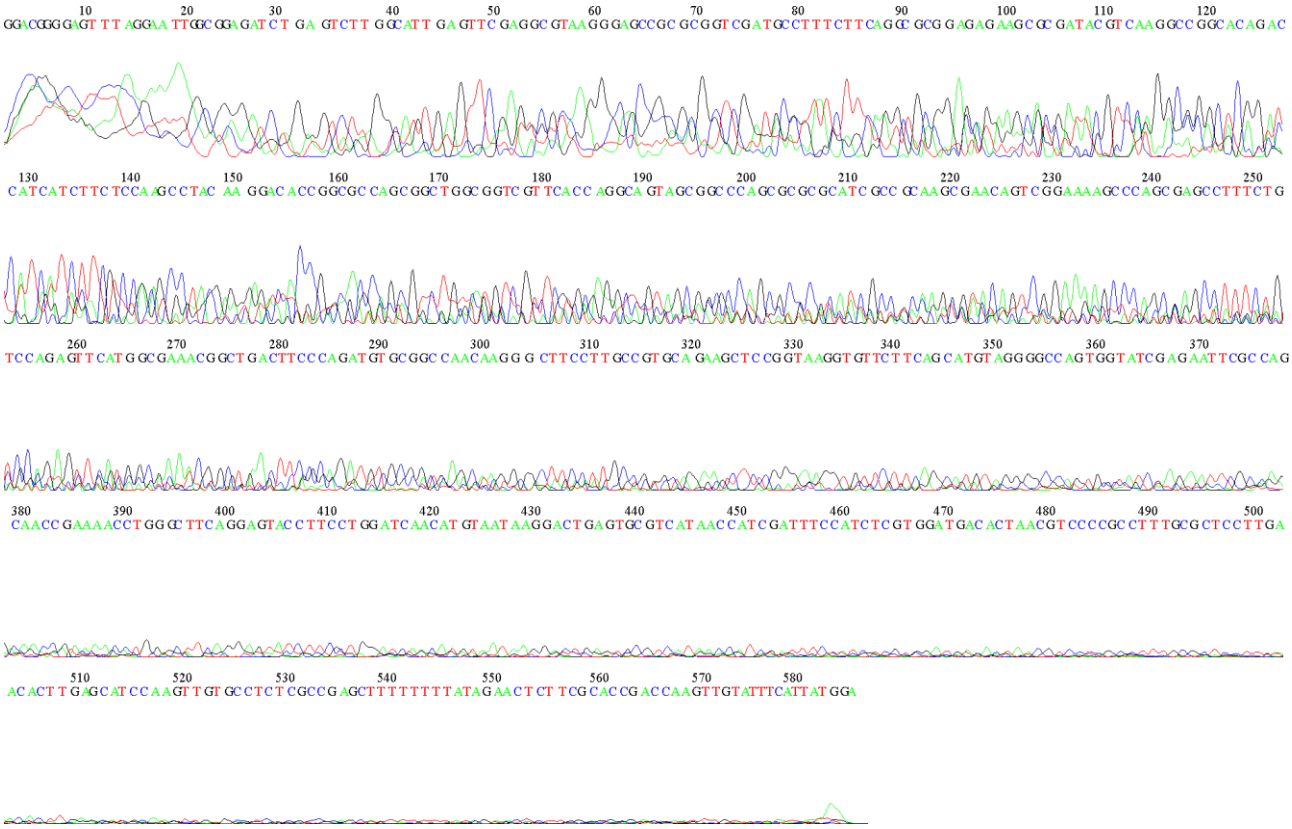
S30	S	<=4	R	>=64	S	<=4	R	>=64	R	>=16	S	16	I	8	R	>=4	R	>=8	*R	4
S31	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S32	S	32	R	>=64	*I	4*	R	>=64	R	8	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S33	S	16	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	I	2	R	>=8	R	>=8
S34	S	16	R	>=64	S	4	I	16	R	8	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S35	S	32	R	>=64	S	>=64	R	>=64	S	>=16	R	>=64	S	>=16	R	>=4	S	>=8	R	>=8
S36	S	16	R	>=64	*I	4*	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S37	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S38	S	16	R	>=64	S	8	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S39	S	16	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S40	S	16	R	>=64	*I	4*	R	>=64	R	8	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S41	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S42	S	<=4	*R	<=4	S	8	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	*R	<=0.5
S43	S	<=4	R	32	S	4	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	*R	<=0.5
S44	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S45	S	<=4	R	>=64	I	16	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	S	1	R	>=8
S46	S	16	R	>=64	S	4	I	16	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S47	S	16	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	I	8	R	>=4	R	>=8	*R	4
S48	S	8	R	>=64	S	2	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S49	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8
S50	S	32	R	>=64	R	>=64	R	>=64	R	>=16	R	>=64	R	>=16	R	>=4	R	>=8	R	>=8

***INP: Interpretation, MIC : Minimum inhibition concentration, R : Resistance, S : Sensitive, I : Intermediate, CZ: Cefazolin, TGC: Tigecycline, IPM: Imipenem, AN: Amikacin, LEV: Levofloxacin, GM: Gentamicin, FEP: Cefepime, CAZ: Ceftazidime, CIP: Ciprofloxacin, TZP: Piperacillin/Tazobactem.**

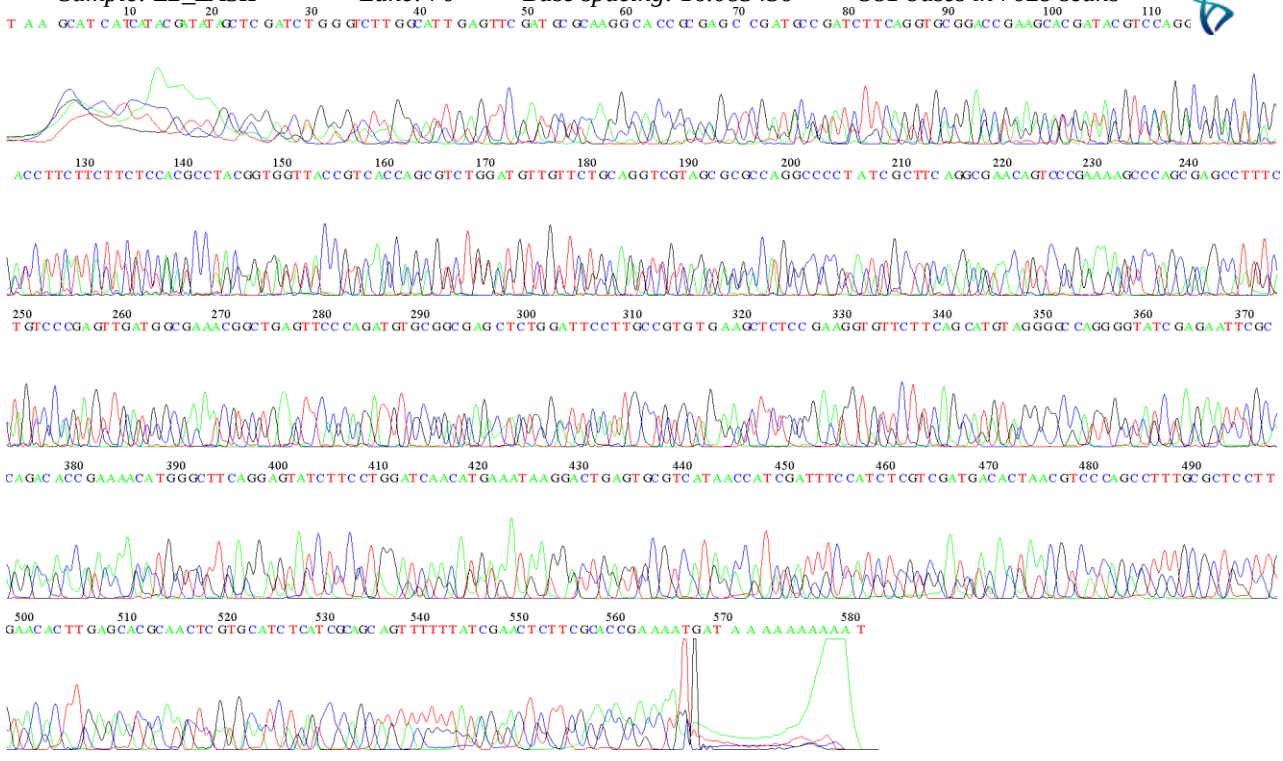
Appendix (2A).

Sequencing analysis for *lasI* gene of *P. aeruginosa* isolated for Z1, Z2 respectively.

***File: Z1**_LASIF.ab1* *Run Ended: 2021/4/6 20:29:41* *Signal G:371 A:450 C:931 T:424*
Sample: Z1_LASIF *Lane: 72* *Base spacing: 16.123789* *587 bases in 7013 scans*



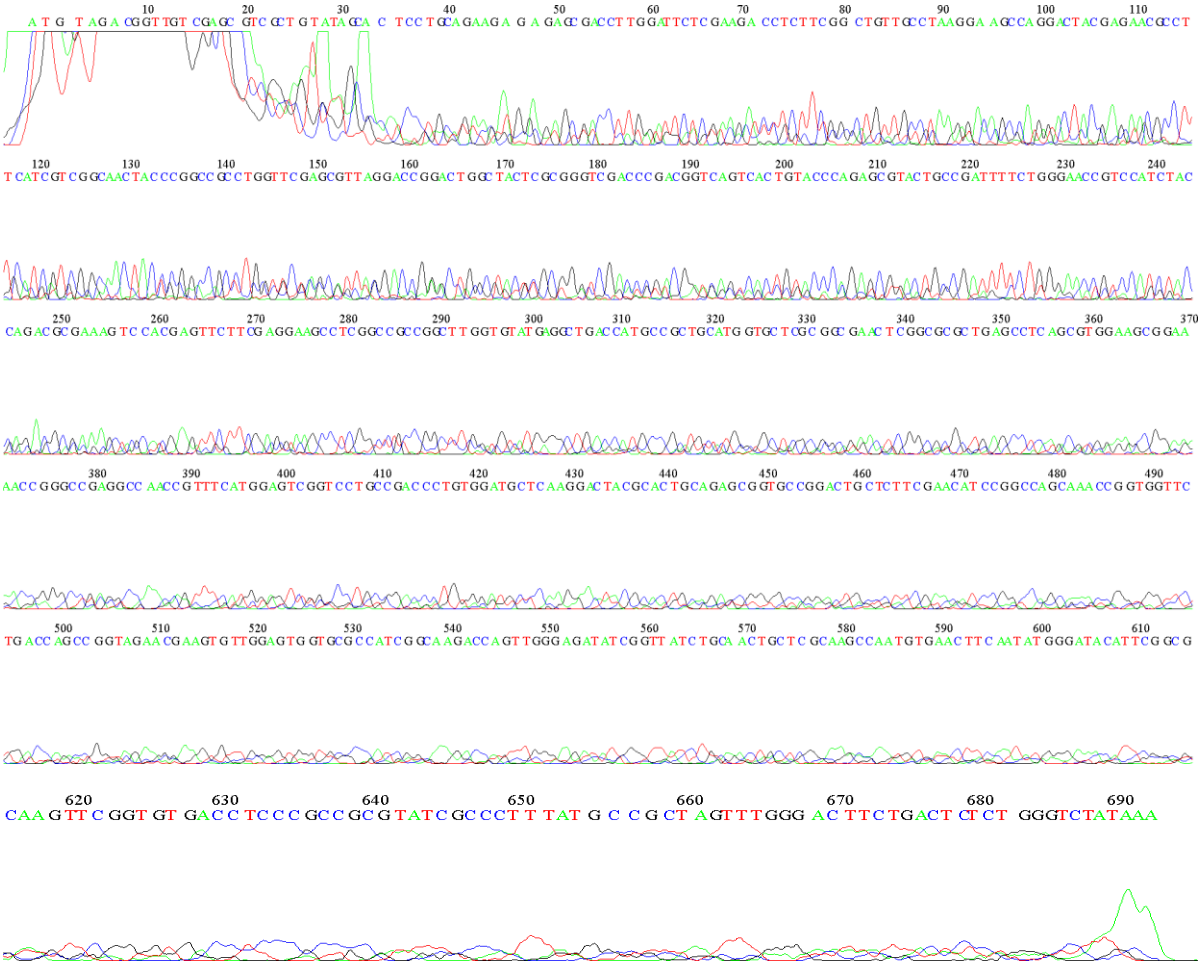
***File: Z2**_LASIF.ab1* *Run Ended: 2021/4/6 20:29:41* *Signal G:618 A:860 C:1852 T:787*
Sample: Z2_LASIF *Lane: 70* *Base spacing: 16.085436* *581 bases in 7029 scans*



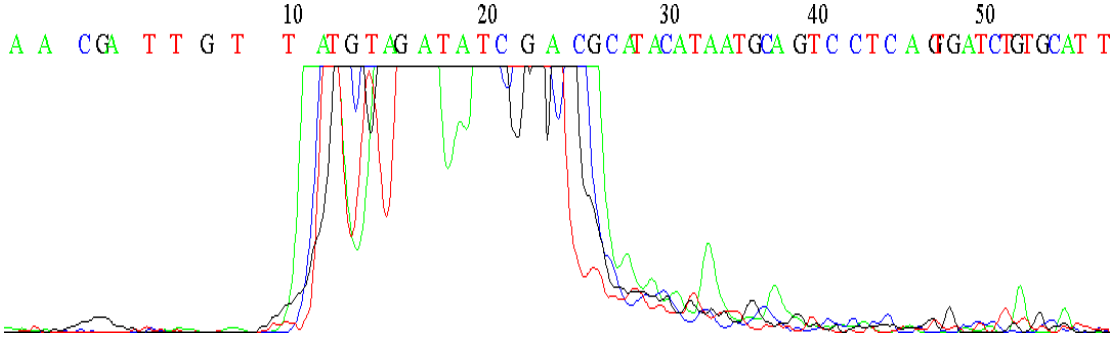
Appendix (2B).

Sequencing analysis for *lasR* gene of *P. aeruginosa* isolated for Y1, Y2 respectively.

***File: Y1**_LASRF.ab1 Run Ended: 2021/4/6 20:29:41 Signal G:164 A:330 C:382 T:218*
Sample: Y1_LASRF Lane: 71 Base spacing: 15.866383 693 bases in 8496 scans



***File: Y2**_LASRF.ab1 Run Ended: 2021/4/6 20:29:41 Signal G:731 A:4379 C:2378 T:1391*
Sample: Y2_LASRF Lane: 69Base spacing: -16.163063 59 bases in 831 scans

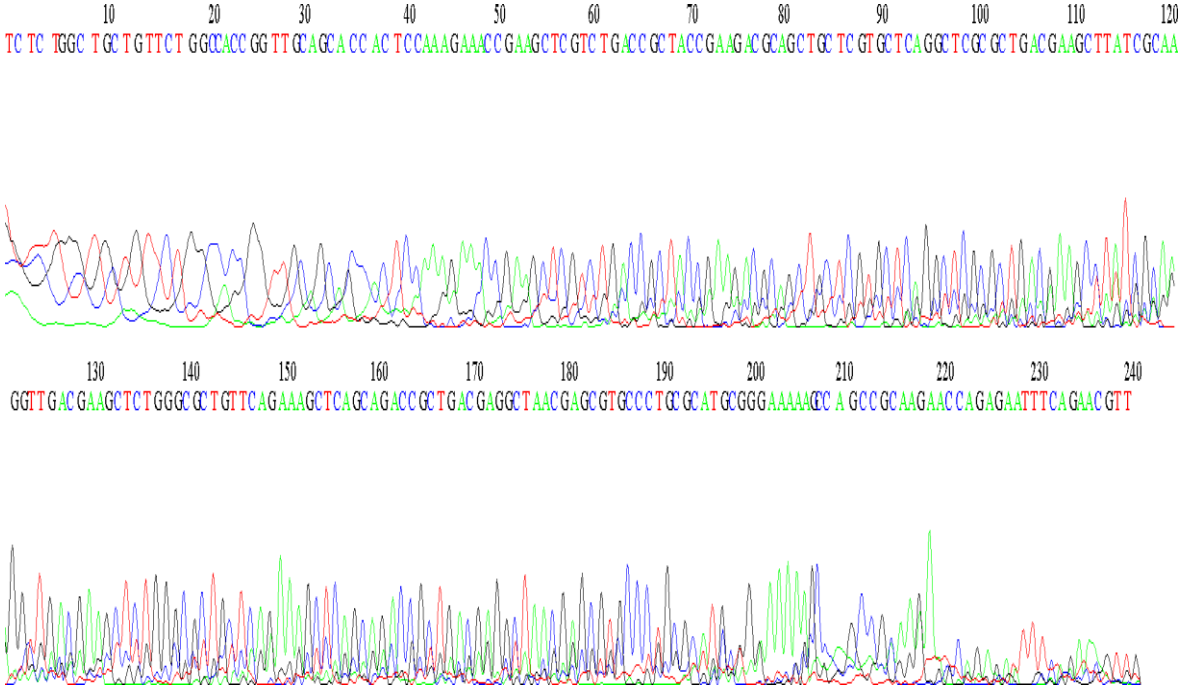


Appendix (2C).

Sequencing analysis for *oprI* gene of *P. aeruginosa* isolated for D1, D2 respectively.

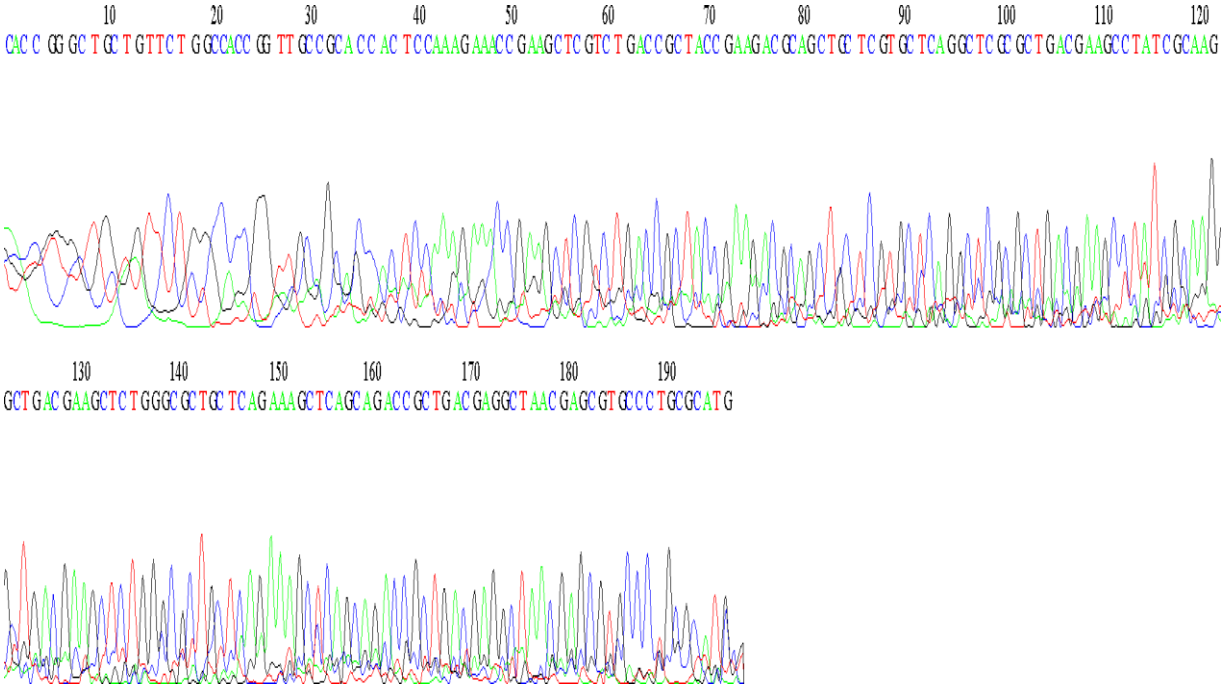
***File: D1**_OPRIF.ab1* *Run Ended: 2021/4/6 19:4:37* *Signal G:1058 A:1986 C:4486 T:1224*

Sample: D1_OPRIF *Lane: 52* *Base spacing: 13.201505* *240 bases in 2956 scans*



***File: D2**_OPRIF.ab1* *Run Ended: 2021/4/6 19:4:37* *Signal G:825 A:1637 C:3935 T:1133*

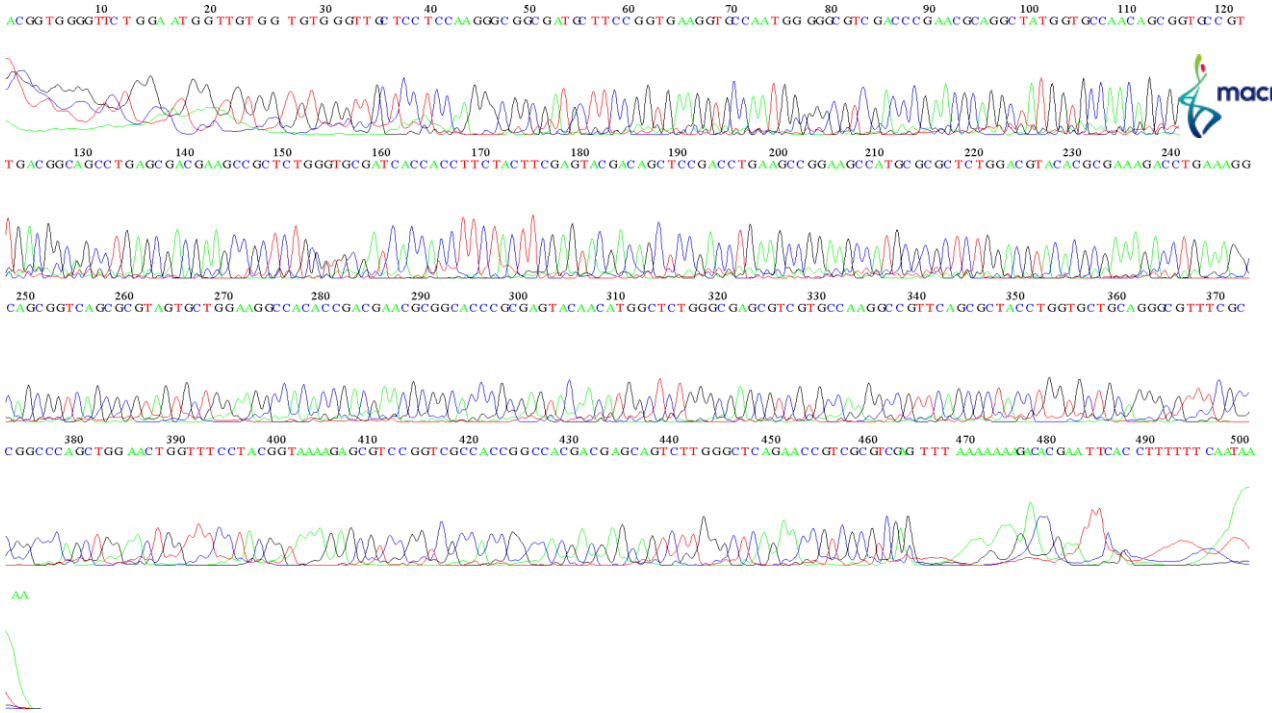
Sample: D2_OPRIF *Lane: 50* *Base spacing: 13.422589* *197 bases in 2412 scans*



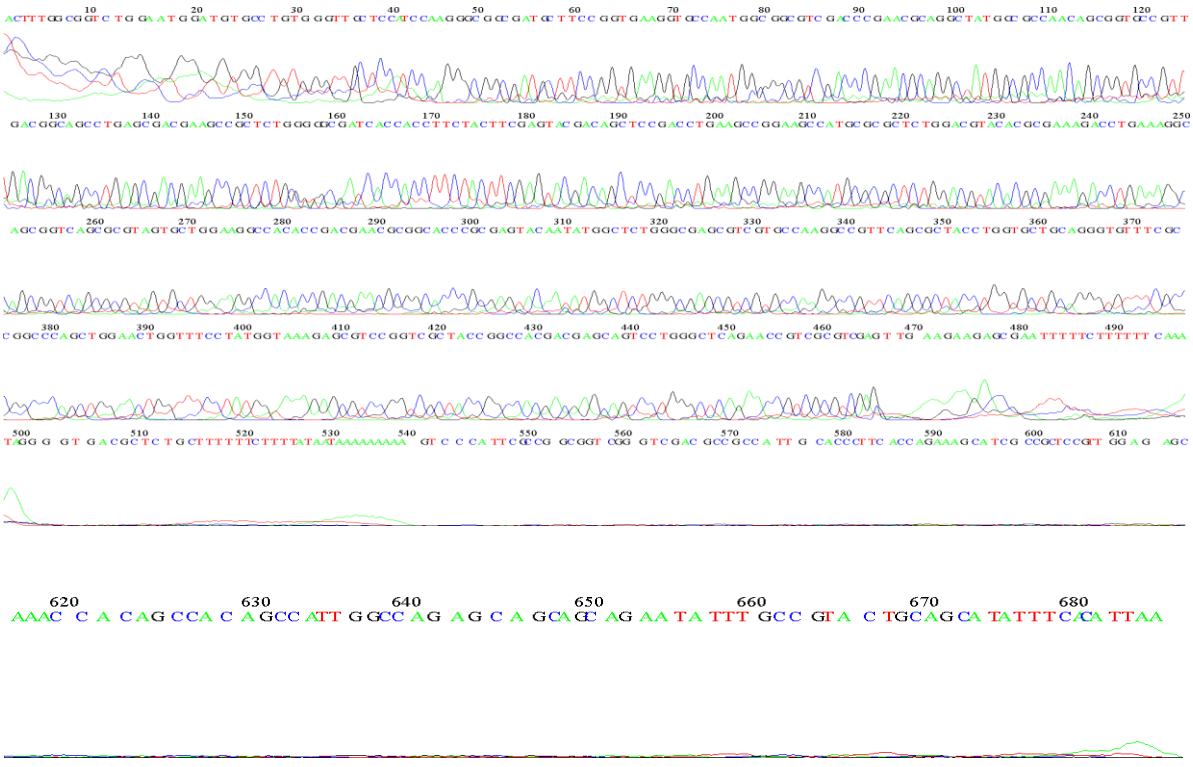
Appendix (2D).

Sequencing analysis for *oprL* gene of *P. aeruginosa* isolated for W1, W2 respectively.

***File: W1**_OPRLF.ab1* *Run Ended: 2021/4/6 19:4:37* *Signal G:558 A:885 C:2611 T:638*
Sample: W1_OPRLF *Lane: 51* *Base spacing: 13.273209* *504 bases in 6042 scans*



***File: W2**_OPRLF.ab1* *Run Ended: 2021/4/6 19:4:37* *Signal G:344 A:655 C:1704 T:464*
Sample: W2_OPRLF *Lane: 49* *Base spacing: 13.349827* *687 bases in 8403 scans*



Appendix (3).

Sequencing analysis / Alignment of *lasI* gene of *P. aeruginosa* isolated for Z1, Z2 samples respectively.

***Pseudomonas aeruginosa* strain PA0750 chromosome, complete genome**
Sequence ID: CP034908.2 Length: 6241875 Number of Matches: 1
Range 1: 1554805 to 1555355GenBankGraphics

Alignment statistics for Z1

Score	Expect	Identities	Gaps	Strand
728 bits(394)	**0.0**	**499/551(91%)**	**2/551(0%)**	**Plus/Minus**

```
Query  27       GATCTGAGTCTTGGCATTGAGTTCGAGGCGTAAGGGAGCCGCGCGGTCGATGCCTTTCTT  86
                |||||| |||||||||||||||||| ||| ||||   ||||||| ||||||||  ||||
Sbjct  1555355  GATCTGGGTCTTGGCATTGAGTTCGATGCGCAAGGCCACCGCGCGCTCGATGCCGATCTT  1555296
Query  87       CAGGCGCGGAGAGAAGCGCGATACGTCAAGGCCGGCACAGACCATCATCTTCTCCAAGCC  146
                |||| |||||  ||||||||||||||| |||||||||| || ||||||||||||||| |||
Sbjct  1555295  CAGGTGCGGACCGAAGCGCGATACGTCCAGGCCGGCACGGATCATCATCTTCTCCACGCC  1555236
Query  147      TAC-AAGGACACCGGCGCCAGCGGCTGGCGGTCGTTCACCAGGCAGTAGCGGCCCAGCGC  205
                |||   ||  |||| | |||||| ||||  |||||||  ||||| ||||||| |||||||
Sbjct  1555235  TACGGTGGTTACCGTCACCAGCGTCTGGATGTCGTTCTGCAGGCTGTAGCGGGCCAGCGC  1555176
Query  206      GCGCATCGCCGCAAGCGAACAGTCGGAAAAGCCCAGCGAGCCTTTCTGTCCAGAGTTCAT  265
                |||||||||| | |||| ||||||||||||||||||||||||||||||||||||||| ||
Sbjct  1555175  GCGCATCGCCTCCAGCGTACAGTCGGAAAAGCCCAGCGAGCCTTTCTGTCCAGAGTTGAT  1555116
Query  266      GGCGAAACGGCTGACTTCCCAGATGTGCGGCCAACAAGG-GCTTCCTTGCCGTGCAGAAG  324
                |||||||||||||| ||||||||||||||| | ||||| ||||||||||||||||||||
Sbjct  1555115  GGCGAAACGGCTGAGTTCCCAGATGTGCGGCGAGCAAGGCGCTTCCTTGCCGTGCAGAAG  1555056
Query  325      CTCCGGTAAGGTGTTCTTCAGCATGTAGGGGCCAGTGGTATCGAGAATTCGCCAGCAACC  384
                |||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1555055  CTCCGGGAAGGTGTTCTTCAGCATGTAGGGGCCAGTGGTATCGAGAATTCGCCAGCAACC  1554996
Query  385      GAAAACCTGGGCTTCAGGAGTACCTTCCTGGATCAACATGTAATAAGGACTGAGTGCGTC  444
                |||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  1554995  GAAAACCTGGGCTTCAGGAGTATCTTCCTGGATCAACATGTAATAAGGACTGAGTGCGTC  1554936
Query  445      ATAACCATCGATTTCCATCTCGTGGATGACACTAACGTCCCCGCCTTTGCGCTCCTTGAA  504
                |||||||||||||||||||||| |||||||||||||||| ||||||||||||||||||||
Sbjct  1554935  ATAACCATCGATTTCCATCTCGTCGATGACACTAACGTCCCAGCCTTTGCGCTCCTTGAA  1554876
Query  505      CACTTGAGCATCCAAGTTGTGCCTCTCGCCGAGCtttttttttATAGAACTCTTCGCACCG  564
                |||||||||  ||| |||||| ||||||| |||  |||||||| ||||||||| |||
Sbjct  1554875  CACTTGAGCACGCAACTTGTGCATCTCGCCCAGCAGTTTTTTATCGAACTCTTCGCGCCG  1554816
Query  565      ACCAAGTTGTA  575
                |||||  |||||
Sbjct  1554815  ACCAATTTGTA  1554805
```

***Pseudomonas aeruginosa* strain CDN118 chromosome, complete genome**
Sequence ID: CP054591.1Length: 6832395 Number of Matches: 1
Range 1: 2273925 to 2274470GenBankGraphics

Alignment statistics for Z2

Score	Expect	Identities	Gaps	Strand
761 bits(412)	**0.0**	**504/548(92%)**	**7/548(1%)**	**Plus/Minus**

```
Query  21       AGCTCGATCTGGGTCTTGGCATTGAGTTCGATGCGCAAGGC-ACCGCGAGC-CGATGCCG  78
                ||| ||||||||||||||||||||||||||||||||||||| |||||| || ||||||||
Sbjct  2274470  AGCGCGATCTGGGTCTTGGCATTGAGTTCGATGCGCAAGGCCACCGCGCGCTCGATGCCG  2274411
Query  79       ATCTTCAGGTGCGGACCGAAGCACGATACGTCCAGGCCGGCTCGGACCTTCTTCTTCTCC  138
                |||||||||||||||||||||| |||||||||||||||| |||| | || ||||||||
Sbjct  2274410  ATCTTCAGGTGCGGACCGAAGCGCGATACGTCCAGGCCGGCACGGATCATCATCTTCTCC  2274351
Query  139      ACGCCTACGGTGGTTACCGTCACCAGCGTCTGGATGTTGTTCTGCAGGTCGTAGCGCGCC  198
                |||||||||||||||||||||||||||||||||||||| ||||||||||  |||||| |||
Sbjct  2274350  ACGCCTACGGTGGTTACCGTCACCAGCGTCTGGATGTCGTTCTGCAGGCTGTAGCGGGCC  2274291
Query  199      AG-GCCCCTATCGCTTCAGGCGAACAGTCCCGAAAAGCCCAGCGAGCCTTTCTGTCCCGA  257
                || || |  ||||| ||  ||| ||||| | ||||||||||||||||||||||||||||
Sbjct  2274290  AGCGCGCGCATCGCCTCCAGCGTACAGT-CGGAAAAGCCCAGCGAGCCTTTCTGTCCAGA  2274232
Query  258      GTTGATGGCGAAACGGCTGAGTTCCCAGATGTGCGGCGAGCTCTG-GATTCCTTGCCGTG  316
                |||||||||||||||||||||||||||||||||||||||||||   | | ||||||||||||
Sbjct  2274231  GTTGATGGCGAAACGGCTGAGTTCCCAGATGTGCGGCGAGCAAGGCGCTTCCTTGCCGTG  2274172
Query  317      -TGAAGCTCTCCGAAGGTGTTCTTCAGCATGTAGGGGCCAGGGGTATCGAGAATTCGCCA  375
                 |||||||   |||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct  2274171  CAGAAGCTCCGGGAAGGTGTTCTTCAGCATGTAGGGGCCAGTGGTATCGAGAATTCGCCA  2274112
Query  376      GACACCGAAAACATGGGCTTCAGGAGTATCTTCCTGGATCAACATGAAATAAGGACTGAG  435
                |  |||||||| ||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  2274111  GCAACCGAAAACCTGGGCTTCAGGAGTATCTTCCTGGATCAACATGTAATAAGGACTGAG  2274052
```

```
Query  436      TGCGTCATAACCATCGATTTCCATCTCGTCGATGACACTAACGTCCCAGCCTTTGCGCTC  495
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2274051  TGCGTCATAACCATCGATTTCCATCTCGTCGATGACACTAACGTCCCAGCCTTTGCGCTC  2273992
Query  496      CTTGAACACTTGAGCACGCAACTCGTGCATCTCATCGCAGCAGTTTTTTATCGAACTCTT  555
                |||||||||||||||||||||| |||||||||  | ||||||||||||||||||||||||
Sbjct  2273991  CTTGAACACTTGAGCACGCAACTTGTGCATCTCGCC-CAGCAGTTTTTTATCGAACTCTT  2273933
Query  556      CGCACCGA  563
                ||| ||||
Sbjct  2273932  CGCGCCGA  2273925
```

Appendix (4).

Sequencing analysis / Alignment of *lasR* gene of *P. aeruginosa* isolated for Y1, Z3 samples respectively.

***Pseudomonas aeruginosa* strain ST773 chromosome, complete genome**
Sequence ID: CP041945.1Length: 6835731 Number of Matches: 1
Range 1: 4280456 to 4281110GenBankGraphics

Alignment statistics for Y1

Score	Expect	Identities	Gaps	Strand
1005 bits(544)	**0.0**	**620/656(95%)**	**8/656(1%)**	**Plus/Minus**

```
Query  35       TCCTGCAGAAGA--GAGAGCGACCTTGGATTCTCGAAGA-CCTCTTCGG-CTGTTGCCTA  90
                ||||||||||||  | ||||||||||||||||||||||| ||||||||| ||||||||||
Sbjct  4281110  TCCTGCAGAAGATGGCGAGCGACCTTGGATTCTCGAAGATCCTCTTCGGCCTGTTGCCTA  4281051
Query  91       AGGA-AGCCAGGACTACGAGAACGCCTTCATCGTCGGCAACTACCCGGCCGCCTGGTTCG  149
                ||||  ||||||||||||||||||||||||||||||||||||||||||||||||||  ||
Sbjct  4281050  AGGACAGCCAGGACTACGAGAACGCCTTCATCGTCGGCAACTACCCGGCCGCCTGGCGCG  4280991
Query  150      AGCGTTAGGACCGGACTGGCTACTCGCGGGTCGACCCGACGGTCAGTCACTGTACCCAGA  209
                ||| ||| |||||| |||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  4280990  AGCATTACGACCGGGCTGGCTACGCGCGGGTCGACCCGACGGTCAGTCACTGTACCCAGA  4280931
Query  210      GCGTACTGCCGATTTTCTGGGAACCGTCCATCTACCAGACGCGAAAGTC-CACGAGTTCT  268
                ||||||||||||||||||||||||||||||||||||||||||||||||   ||||||||||
Sbjct  4280930  GCGTACTGCCGATTTTCTGGGAACCGTCCATCTACCAGACGCGAAAGCAGCACGAGTTCT  4280871
Query  269      TCGAGGAAGCCTCGGCCGCCGGCTTGGTGTATGAGGCTGACCATGCCGCTGCATGGTGCT  328
                |||||||||||||||||||| ||||||||| ||||||||||||||||||||||||||||
Sbjct  4280870  TCGAGGAAGCCTCGGCCGCCGGCCTGGTGTATG-GGCTGACCATGCCGCTGCATGGTGCT  4280812
Query  329      CGCGGCGAACTCGGCGCGCTGAGCCTCAGCGTGGAAGCGGAAAACCGGGCCGAGGCCAAC  388
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4280811  CGCGGCGAACTCGGCGCGCTGAGCCTCAGCGTGGAAGCGGAAAACCGGGCCGAGGCCAAC  4280752
Query  389      CGTTTCATGGAGTCGGTCCTGCCGACCCTGTGGATGCTCAAGGACTACGCACTGCAGAGC  448
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4280751  CGTTTCATGGAGTCGGTCCTGCCGACCCTGTGGATGCTCAAGGACTACGCACTGCAGAGC  4280692
Query  449      GGTGCCGGACTGCTCTTCGAACATCCGGCCAGCAAACCGGTGGTTCTGACCAGCCGGTAG  508
                ||||||||||||  |||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  4280691  GGTGCCGGACTGGCCTTCGAACATCCGGTCAGCAAACCGGTGGTTCTGACCAGCCGGGAG  4280632
Query  509      AACGAAGTGTTGGAGTGGTGCGCCATCGGCAAGACCAGTTGGGAGATATCGGTTATCTGC  568
                || ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4280631  AAGGAAGTGTTGCAGTGGTGCGCCATCGGCAAGACCAGTTGGGAGATATCGGTTATCTGC  4280572
Query  569      AACTGCTCGCAAGCCAATGTGAACTTCAATATGGGATACATTCGGCGCAAGTTCGGTGTG  628
                ||||||||| ||||||||||||||| |||||||| |||||||||| ||||||||||||||
Sbjct  4280571  AACTGCTCGGAAGCCAATGTGAACTTCCATATGGGAAACATTCGGCGGAAGTTCGGTGTG  4280512
Query  629      ACCTCCCGCCGCGTATCGCCCTTTATG-CCGCTAGTTTGGGACTTCTGACTCTCTG  683
                |||||||||||||| || || ||||| ||| || |||||| ||| | ||||||||
Sbjct  4280511  ACCTCCCGCCGCGTAGCGGCCATTATGGCCGTTAATTTGGGTCTTATTACTCTCTG  4280456
```

***Pseudomonas aeruginosa* strain PA_154197, complete genome**
Sequence ID: CP014866.1Length: 6445239 Number of Matches: 1
Range 1: 4047602 to 4048273GenBankGraphics

Alignment statistics for Y3

Score	Expect	Identities	Gaps	Strand
837 bits(453)	**0.0**	**603/676(89%)**	**7/676(1%)**	**Plus/Minus**

```
Query  24       ATTGGAGTGGAGC-CCATCCTGCAGAACATGGAGAGCGACCTTGGATTCTCGAAGATCTT  82
                |||||||||||||| |||||||||||||| |||| |||||||||||||||||||||||| |
Sbjct  4048273  ATTGGAGTGGAGCGCCATCCTGCAGAAGATGGCGAGCGACCTTGGATTCTCGAAGATCCT  4048214
Query  83       GTTCGGCCTGTTGCCTAAGGACAGCCAGGACTACGAGAACGCCTTCATCGTCGGCAACTA  142
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4048213  GTTCGGCCTGTTGCCTAAGGACAGCCAGGACTACGAGAACGCCTTCATCGTCGGCAACTA  4048154
Query  143      CCCGGCCGCCTGGTTCTAGCGTTACGACCGAACTGGCTACGCGCGGGTCGACCCGACGAA  202
                ||||||||||||  | ||| |||||||||  |||||||||||||||||||||||||||||
Sbjct  4048153  CCCGGCCGCCTGGCGCGAGCATTACGACCGGGCTGGCTACGCGCGGGTCGACCCGACGGT  4048094
Query  203      GAGTCACTGTACCCCGAGCGTACTGCCGATTTTCTGGGAACCGTCCATCTACCAGACGCG  262
```

```
                 |||||||||||||  ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4048093  CAGTCACTGTACCCAGAGCGTACTGCCGATTTTCTGGGAACCGTCCATCTACCAGACGCG  4048034
Query  263      AAAGCC-CACGAGTTCTTCGAGGAAGCCTCGGCCGCCGGCTTGGTGTATGAGGCTGACCA  321
                 ||||||  ||||||||||||||||||||||||||||||||  |||||||||  |||||||||
Sbjct  4048033  AAAGCCGCACGAGTTCTTCGAGGAAGCCTCGGCCGCCGGCCTGGTGTATG-GGCTGACCA  4047975
Query  322      TGCCGCTGCATGCCGCTCGCGGAGAACTCGGCGCGCTGAGCCTCAGCGCGGAAGCGGAGA  381
                 ||||||||||||  ||||||||  |||||||||||||||||||||||||  |||||||||  |
Sbjct  4047974  TGCCGCTGCATGGTGCTCGCGGCGAACTCGGCGCGCTGAGCCTCAGCGTGGAAGCGGAAA  4047915
Query  382      ACCGGGCCGAGGCCAACCGTTTCATGGAGTCGGTCCTGCGGACCATGTGGATGCCCAAGA  441
                 ||||||||||||||||||||||||||||||||||||||||||||||||  ||||  |||||||||  ||||
Sbjct  4047914  ACCGGGCCGAGGCCAACCGTTTCATGGAGTCGGTCCTGCCGACCCTGTGGATGCTCAAGG  4047855
Query  442      ACTACGCACTGACGAGCGGGGCCGAATTGCTCTTCGAACATCCGACCAGCAAACAGCTGG  501
                 ||||||||||  ||||||  ||||  |  ||  |||||||||||||  ||||||||  |  |||
Sbjct  4047854  ACTACGCACTGCAGAGCGGTGCCGGACTGGCCTTCGAACATCCGGTCAGCAAACCGGTGG  4047795
Query  502      TTCTGACCAGCCGCTAGAACAAAGCGTTGCAGTGGTGCGCCATCGGCAAGACCAGCTCGT  561
                 ||||||||||||  ||||  |||  ||||||||||||||||||||||||||||||  |  |
Sbjct  4047794  TTCTGACCAGCCGGGAGAAGGAAGTGTTGCAGTGGTGCGCCATCGGCAAGACCAGTTGGG  4047735
Query  562      AGACATCGCTTATCTGCAACTGCTCGCAAGCCAATGTCAACTTCAATATAAGATCTATCC  621
                 |||  ||||  ||||||||||||||||  ||||||||||  ||||||  ||||  ||  |||  |
Sbjct  4047734  AGATATCGGTTATCTGCAACTGCTCGGAAGCCAATGTGAACTTCCATATGGGAAATATTC  4047675
Query  622      GCAGCAAGTTCGGTATGACCTTCCCGCTGCATATCGCCAATTATG-CCGTTCTAGTTCGG  680
                 |  |  |||||||||  ||||||  |||||  ||  ||  ||  |  ||||||  |||||  |  ||  ||
Sbjct  4047674  GGCGGAAGTTCGGTGTGACCT-CCCGCCGCGTAGCGGCCATTATGGCCGTTA-ATTT-GG  4047618
Query  681      GTCTTATAACGCACTG  696
                 |||||||  ||  |  |||
Sbjct  4047617  GTCTTATTACTCTCTG  4047602
```

Appendix (5).

Sequencing analysis / Alignment of *oprI* gene of *P. aeruginosa* isolated for D1, Z2 samples respectively.

***Pseudomonas aeruginosa* strain DVT401 chromosome, complete genome**
Sequence ID: CP050335.1Length: 6557595Number of Matches: 1
Range 1: 2300830 to 2301047GenBankGraphics

Alignment statistics for D1

Score	Expect	Identities	Gaps	Strand
374 bits(202)	**1e-101**	**213/218(98%)**	**1/218(0%)**	**Plus/Minus**

```
Query  2        CTCTGGCTGCTGTTCTGGCCACCGGTTGCAGCA-CCACTCCAAAGAAACCGAAGCTCGTC  60
                ||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct  2301047  CTCTGGCTGCTGTTCTGGCCACCGGTTGCAGCAGCCACTCCAAAGAAACCGAAGCTCGTC  2300988
Query  61       TGACCGCTACCGAAGACGCAGCTGCTCGTGCTCAGGCTCGCGCTGACGAAGCTTATCGCA  120
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct  2300987  TGACCGCTACCGAAGACGCAGCTGCTCGTGCTCAGGCTCGCGCTGACGAAGCCTATCGCA  2300928
Query  121      AGGTTGACGAAGCTCTGGGCGCTGTTCAGAAAGCTCAGCAGACCGCTGACGAGGCTAACG  180
                ||| |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  2300927  AGGCTGACGAAGCTCTGGGCGCTGCTCAGAAAGCTCAGCAGACCGCTGACGAGGCTAACG  2300868
Query  181      AGCGTGCCCTGCGCATGCGGGAAAAAGCCAGCCGCAAG  218
                ||||||||||||||||||| ||||||||||||||||||||
Sbjct  2300867  AGCGTGCCCTGCGCATGCTGGAAAAAGCCAGCCGCAAG  2300830
```

***Pseudomonas aeruginosa* strain DVT401 chromosome, complete genome**
Sequence ID: CP050335.1Length: 6557595 Number of Matches: 1
Range 1: 2300851 to 2301043GenBankGraphics

Alignment statistics for D2

Score	Expect	Identities	Gaps	Strand
344 bits(186)	**9e-93**	**191/193(99%)**	**1/193(0%)**	**Plus/Minus**

```
Query  6        GGCTGCTGTTCTGGCCACCGGTTGCCGCA-CCACTCCAAAGAAACCGAAGCTCGTCTGAC  64
                ||||||||||||||||||||||||| ||| ||||||||||||||||||||||||||||||
Sbjct  2301043  GGCTGCTGTTCTGGCCACCGGTTGCAGCAGCCACTCCAAAGAAACCGAAGCTCGTCTGAC  2300984
Query  65       CGCTACCGAAGACGCAGCTGCTCGTGCTCAGGCTCGCGCTGACGAAGCCTATCGCAAGGC  124
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2300983  CGCTACCGAAGACGCAGCTGCTCGTGCTCAGGCTCGCGCTGACGAAGCCTATCGCAAGGC  2300924
Query  125      TGACGAAGCTCTGGGCGCTGCTCAGAAAGCTCAGCAGACCGCTGACGAGGCTAACGAGCG  184
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2300923  TGACGAAGCTCTGGGCGCTGCTCAGAAAGCTCAGCAGACCGCTGACGAGGCTAACGAGCG  2300864
Query  185      TGCCCTGCGCATG  197
                |||||||||||||
Sbjct  2300863  TGCCCTGCGCATG  2300851
```

Appendix (6).

Sequencing analysis / Alignment of *oprL* gene of *P. aeruginosa* isolated for W1, W2 samples respectively.

***Pseudomonas aeruginosa* strain PSE6684 chromosome, complete genome**
Sequence ID: CP053917.1Length: 6924367 Number of Matches: 1
Range 1: 4845520 to 4845979GenBankGraphics

Alignment statistics for W1

Score	Expect	Identities	Gaps	Strand
804 bits(435)	**0.0**	**453/461(98%)**	**3/461(0%)**	**Plus/Plus**

```
Query  8        GGTTCTGG-AATGGTTGTGG-TGTGGGTTGCTCCTCCAAGGGCGGCGATGCTTCCGGTGA  65
               || |||||  |||| ||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  4845520  GGCTCTGGCCATGGCTGTGGCTGTGGGTTGCTCCTCCAAGGGCGGCGATGCTTCCGGTGA  4845579
Query  66       AGGTGCCAATGGGGGCGTCGACCCGAACGCAGGCTATGGTGCCAACAGCGGTGCCGTTGA  125
               ||||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4845580  AGGTGCCAATGGCGGCGTCGACCCGAACGCAGGCTATGGTGCCAACAGCGGTGCCGTTGA  4845639
Query  126      CGGCAGCCTGAGCGACGAAGCCGCTCTGGGTGCGATCACCACCTTCTACTTCGAGTACGA  185
               |||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  4845640  CGGCAGCCTGAGCGACGAAGCCGCTCTGCGTGCGATCACCACCTTCTACTTCGAGTACGA  4845699
Query  186      CAGCTCCGACCTGAAGCCGGAAGCCATGCGCGCTCTGGACGTACACGCGAAAGACCTGAA  245
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4845700  CAGCTCCGACCTGAAGCCGGAAGCCATGCGCGCTCTGGACGTACACGCGAAAGACCTGAA  4845759
Query  246      AGGCAGCGGTCAGCGCGTAGTGCTGGAAGGCCACACCGACGAACGCGGCACCCGCGAGTA  305
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4845760  AGGCAGCGGTCAGCGCGTAGTGCTGGAAGGCCACACCGACGAACGCGGCACCCGCGAGTA  4845819
Query  306      CAACATGGCTCTGGGCGAGCGTCGTGCCAAGGCCGTTCAGCGCTACCTGGTGCTGCAGGG  365
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4845820  CAACATGGCTCTGGGCGAGCGTCGTGCCAAGGCCGTTCAGCGCTACCTGGTGCTGCAGGG  4845879
Query  366      CGTTTCGCCGGCCCAGCTGGAACTGGTTTCCTACGGTAAAAGAGCGTCCGGTCGCCACCG  425
               |||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct  4845880  CGTTTCGCCGGCCCAGCTGGAACTGGTTTCCTACGGT-AAAGAGCGTCCGGTCGCCACCG  4845938
Query  426      GCCACGACGAGCAGTCTTGGGCTCAGAACCGTCGCGTCGAG  466
               |||||||||||||||||||||||||||||||||||||||||
Sbjct  4845939  GCCACGACGAGCAGTCTTGGGCTCAGAACCGTCGCGTCGAG  4845979
```

***Pseudomonas aeruginosa* strain PA0750 chromosome, complete genome**
Sequence ID: CP034908.2Length: 6241875 Number of Matches: 1
Range 1: 1052978 to 1053443GenBankGraphics

Alignment statistics for W2

Score	Expect	Identities	Gaps	Strand
817 bits(442)	**0.0**	**459/467(98%)**	**2/467(0%)**	**Plus/Plus**

```
Query  11       TCTGG-AATGGATGTGCCTGTGGGTTGCTCCATCCAAGGGCGGCGATGCTTCCGGTGAAG  69
               |||||  |||| |||| |||||||||||||| ||||||||||||||||||||||||||||
Sbjct  1052978  TCTGGCCATGGCTGTGGCTGTGGGTTGCTCC-TCCAAGGGCGGCGATGCTTCCGGTGAAG  1053036
Query  70       GTGCCAATGGCGGCGTCGACCCGAACGCAGGCTATGGCGCCAACAGCGGTGCCGTTGACG  129
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1053037  GTGCCAATGGCGGCGTCGACCCGAACGCAGGCTATGGCGCCAACAGCGGTGCCGTTGACG  1053096
Query  130      GCAGCCTGAGCGACGAAGCCGCTCTGGGGGCGATCACCACCTTCTACTTCGAGTACGACA  189
               |||||||||||||||||||||||||| | ||||||||||||||||||||||||||||||
Sbjct  1053097  GCAGCCTGAGCGACGAAGCCGCTCTGCGTGCGATCACCACCTTCTACTTCGAGTACGACA  1053156
Query  190      GCTCCGACCTGAAGCCGGAAGCCATGCGCGCTCTGGACGTACACGCGAAAGACCTGAAAG  249
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1053157  GCTCCGACCTGAAGCCGGAAGCCATGCGCGCTCTGGACGTACACGCGAAAGACCTGAAAG  1053216
Query  250      GCAGCGGTCAGCGCGTAGTGCTGGAAGGCCACACCGACGAACGCGGCACCCGCGAGTACA  309
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1053217  GCAGCGGTCAGCGCGTAGTGCTGGAAGGCCACACCGACGAACGCGGCACCCGCGAGTACA  1053276
Query  310      ATATGGCTCTGGGCGAGCGTCGTGCCAAGGCCGTTCAGCGCTACCTGGTGCTGCAGGGTG  369
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1053277  ATATGGCTCTGGGCGAGCGTCGTGCCAAGGCCGTTCAGCGCTACCTGGTGCTGCAGGGTG  1053336
Query  370      TTTCGCCGGCCCAGCTGGAACTGGTTTCCTATGGTAAAGAGCGTCCGGTCGCTACCGGCC  429
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1053337  TTTCGCCGGCCCAGCTGGAACTGGTTTCCTATGGTAAAGAGCGTCCGGTCGCTACCGGCC  1053396
Query  430      ACGACGAGCAGTCCTGGGCTCAGAACCGTCGCGTCGAGTTGAAGAAG  476
               |||||||||||||||||||||||||||||||||||||||| ||||||||
Sbjct  1053397  ACGACGAGCAGTCCTGGGCTCAGAACCGTCGCGTCGAGCTGAAGAAG  1053443
```

Appendix (7).

Type of mutation in the *lasI* gene sequence in *p. aeruginosa* bacteria isolates isolated for Z1, Z2, Z3, Z4, Z5, Z6, Z7, Z8 samples respectively.

NO. of Sample	City	Cause of Burn	Burn Degree	Antibiotic Susceptibility	Site	Point mutation	Type Mutation			
							Transversion	Transition	Insertion	Deletion
Z1	**Najaf**	**Fire**	**Second**	**Resistance**	33	A > C	+			
					53	G > T	+			
					57	T > C		+		
					62	G > C	+			
					63	A > C	+			
					64	G > A		+		
					72	G > A		+		
					81	T > G	+			
					82	T > A	+			
					91	C > T		+		
					97	G > C	+			
					98	A > C	+			
					114	A > C	+			
					125	A > G		+		
					128	C > T		+		
					143	A > C	+			
					149-150	- > G			+	
					150	A > G		+		
					151	A > T	+			
					154	A > T	+			
					155	C > T		+		
					160	G > T	+			
					162	G > A		+		
					169	G > T	+			
					174	C > A	+			
					175	G > T	+			
					183	A > T	+			
					184	C > G	+			
					190	A > T	+			
					198	C > G	+			
					216	G > T	+			
					218	A > C	+			
					223	A > T	+			
					263	C > G	+			
					280	C > G	+			
					297	C > G	+			
					299	A > G		+		
					304-305	- > C			+	
					331	T > G	+			
					407	C > T		+		
					468	G > C	+			
					486	C > A	+			
					515	T > C		+		
					516	C > G	+			
					520	G > C	+			
					527	C > A	+			
					535	G > C	+			
					539	T > A	+			
					540	T > G	+			
					549	A > C	+			
					561	A > G		+		
					570	G > T	+			
					Total		**37**	**13**	**2**	**0**
Z2	**Najaf**	**Fire**	**Second**	**Sensitive**	24	T > G	+			
					61-62	- > C			+	
					68	A > C	+			
					70-71	- > T			+	
					101	A > G		+		
					120	T > A	+			
					125	C > T		+		
					127	T > A	+			
					130	T > A	+			
					176	T > C		+		

					187	T > C		+		
					188	C > T		+		
					195	C > G	+			
					200-201	- > C			+	
					203	C > G	+			
					205	C > G	+			
					206	T > C		+		
					212	T > C		+		
					215	A > C	+			
					216	G > A		+		
					220	A > T	+			
					226	C > -				+
					228	C > G	+			
					255	C > A	+			
					299	T > A	+			
					300	C > A	+			
					301	T > G	+			
					302-303	- > C			+	
					304	A > C	+			
					316-317	- > C			+	
					317	T > A	+			
					325	T > C		+		
					326	C > G	+			
					327	C > G	+			
					357	G > T	+			
					377	A > C	+			
					378	C > A	+			
					388	A > C	+			
					422	A > T	+			
					519	C > T		+		
					529	A > G		+		
					530	T > C		+		
					532	G > -				+
					559	A > G		+		
					Total		**24**	**13**	**5**	**2**
Z3	**Babel**	**Fire**	**Second**	**Sensitive**	100	G > T	+			
					102	A > G		+		
					110	A > T	+			
					114	C > T		+		
					121	G > C	+			
					126	G > A		+		
					128	A > T	+			
					135-136	- > A			+	
					137	T > A	+			
					139	C > -				+
					141	C > T		+		
					142	C > T		+		
					144	C > A	+			
					148	T > A	+			
					149	A > G		+		
					161	G > T	+			
					168	G > C	+			
					169	C > A	+			
					177	T > G	+			
					185	T > -				+
					187-188	- > G			+	
					189	G > A		+		
					190	G > T	+			
					193	G > T	+			
					200	G > C	+			
					234	A > G		+		
					236	A > -				+
					237	A > -				+
					240	C > T		+		
					240-241	- > T			+	
					242	A > T	+			
					250	C > A	+			
					251	T > A	+			
					252	T > G	+			
					253	T > G	+			
					254	C > A	+			

					255	T > A	+			
					257	T > C		+		
					258	C > G	+			
					273	A > C	+			
					275	A > T	+			
					277	G > T	+			
					279	C > G	+			
					280	T > -				+
					287	C > A	+			
					288	C > G	+			
					290	A > C	+			
					291	G > -				+
					312	T > C		+		
					342	G > A		+		
					344	T > G	+			
					348	C > G	+			
					Total		**31**	**12**	**3**	**6**
Z4	**Karbala**	**liquid**	**Third**	**Resistance**	99	G > A		+		
					105	C > G	+			
					106	G > A		+		
					108	T > A	+			
					112	T > C		+		
					114	T > A	+			
					124	A > G		+		
					125	A > G		+		
					150	A > G		+		
					160	G > T	+			
					161	A > C	+			
					189	T > C		+		
					205-206	- > G			+	
					207	C > G	+			
					212-213	- > C			+	
					302	T > G	+			
					303	G > C	+			
					305	A > C	+			
					379	A > C	+			
					380	C > A	+			
					409	C > T		+		
					267-268	- > A			+	
					271-272	- > T			+	
					575	G > -				+
					Total		**11**	**8**	**4**	**1**
Z5	**Karbala**	**liquid**	**Third**	**Sensitive**	62	G > A		+		
					68	C > G	+			
					69	G > C	+			
					75	T > C		+		
					76	C > T		+		
					85	T > A	+			
					86	C > G	+			
					87-88	- > G			+	
					91	G > -				+
					96	G > C	+			
					100	C > A	+			
					103	A > G		+		
					111	A > T	+			
					121	C > G	+			
					122	G > C	+			
					123	G > A		+		
					127	G > A		+		
					129	A > T	+			
					137-138	- > C			+	
					138	T > A	+			
					140	C > G	+			
					142	C > -				+
					143	C > T		+		
					145	C > A	+			
					150	A > G		+		
					169	G > C	+			
					178	T > G	+			
					185	C > -				+
					186	T > G	+			

					188-189	- > G			+	
					194	G > T	+			
					250	C > A	+			
					251	T > A	+			
					252	T > G	+			
					253	T > G	+			
					254	C > A	+			
					255	T > A	+			
					258	C > G	+			
					266	G > C	+			
					267	A > G		+		
					268	T > C		+		
					269	G > C	+			
					273	A > C	+			
					275	A > T	+			
					336	A > C	+			
					337	G > T	+			
					345	T > G	+			
					346	C > G	+			
					350	A > C	+			
					351	T > G	+			
					354	A > G		+		
					355	G > C	+			
					357	G > C	+			
					360	C > G	+			
					361	A > C	+			
					365	G > C	+			
					366	T > C		+		
					374	A > G		+		
					375	T > C		+		
					376	T > C		+		
					377	C > T		+		
					404	G > C	+			
					406	A > -				+
					409-410	- > A			+	
					416-417	- > A			+	
					419	A > G		+		
					419-420	- > G			+	
					421	C > G	+			
					422	A > G		+		
					424	C > G	+			
					426	G > -				+
					427	G > -				+
					432	A > G		+		
					434	G > T	+			
					437	C > T		+		
					438	T > C		+		
					439	G > C	+			
					446	T > C		+		
					448	A > C	+			
					451	A > G		+		
					454	A > G		+		
					460	T > C		+		
					461	T > G	+			
					465	T > -				+
					467	T > G	+			
					468	C > G	+			
					469	G > T	+			
					471-472	- > G			+	
					474	T > C		+		
					480	T > G	+			
					482	A > T	+			
					485	T > G	+			
					493	T > C		+		
					494	T > G	+			
					495	T > C		+		
					499	C > G	+			
					500-501	- > G			+	
					500-501	- > G			+	
					506	T > C		+		
					510	C > -				+

					511	A > -				+
					516	G > -				+
					531	A > C	+			
					532	T > C		+		
					540	A > C	+			
					547	T > C		+		
					548	T > G	+			
					549	T > G	+			
					550	T > G	+			
					551	A > G		+		
					552	T > G	+			
					559	C > G	+			
					560	T > G	+			
					562	C > G	+			
					Total		**63**	**32**	**9**	**10**
Z6	**Babel**	**Fire**	**Second**	**Sensitive**	151	T > A	+			
					156	G > T	+			
					158	C > A	+			
					163	G > C	+			
					164	C > A	+			
					172	T > G	+			
					180	T > -				+
					182-183	- > G			+	
					188	G > T	+			
					195	G > C	+			
					201	G > C	+			
					229	A > G		+		
					231	A > G		+		
					232	A > C	+			
					233	G > T	+			
					236	A > T	+			
					237-238	- > C			+	
					243	T > A	+			
					244	T > A	+			
					245	T > G	+			
					246	C > G	+			
					247	T > A	+			
					247-248	- > A			+	
					249	T > C		+		
					250	C > G	+			
					258	G > C	+			
					259	A > G		+		
					260	T > C		+		
					161	G > C	+			
					264	G > A		+		
					265	A > C	+			
					277	T > C		+		
					308	T > G	+			
					Total		**22**	**7**	**3**	**1**
Z7	**Babel**	**Fire**	**Second**	**Resistance**	116	G > C	+			
					117	G > A		+		
					121	G > A		+		
					123	A > T	+			
					132	T > C		+		
					133	T > A	+			
					140	G > T	+			
					143	T > A	+			
					152	T > C	+	+		
					158	C > A	+			
					179	C > G	+			
					180	T > G	+			
					188	G > T				
					189	T > C		+		
					190	A > G		+		
					244	T > A	+			
					245	T > A	+			
					246	T > G	+			
					247	C > G	+			
					251	T > C		+		
					252	C > G	+			
					266	G > A		+		

					330	A > C	+			
					334	G > A		+		
					336	T > G	+			
					340	C > G	+			
					344	A > C	+			
					345	T > G	+			
					349	G > C	+			
					362	T > G	+			
					368	A > G		+		
					369	T > C		+		
					370	T > C		+		
					374	C > A	+			
					383	A > T	+			
					398	G > C	+			
					400	A > -				+
					403-404	- > C			+	
					410-411	- > A			+	
					413	A > C	+			
					413-414	- > G			+	
					415	C > G	+			
					416	A > G		+		
					418	C > -				+
					419	A > -				+
					423	A > G		+		
					426	A > G		+		
					428	G > T	+			
					431	C > T		+		
					432	T > C		+		
					440	T > G	+			
					445	A > G		+		
					448	A > G		+		
					454	T > C		+		
					455	T > G	+			
					459	T > C		+		
					460	C > G	+			
					460-461	- > G			+	
					463	G > -				+
					476	A > T	+			
					487	T > C		+		
					493	C > G	+			
					494-495	- > G			+	
					494-495	- > G			+	
					600	A > -				+
					601	A > -				+
					505	T > A	+			
					514	C > A	+			
					Total		**34**	**22**	**6**	**6**
Z8	**Babel**	**Fire**	**Third**	**Sensitive**	98-99	- > T			+	
					102	G > -				+
					106	G > C	+			
					109	A > T	+			
					113	C > T		+		
					119	C > G	+			
					125	G > A		+		
					127	A > T	+			
					134-135	- > A			+	
					136	T > A	+			
					138	C > -				+
					140	C > T		+		
					141	C > T		+		
					143	C > A	+			
					144	G > T	+			
					147	T > A	+			
					157	A > T	+			
					159-160	- > T			+	
					161	G > A		+		
					163	C > G	+			
					166	G > C	+			
					167	C > A	+			
					173	G > T	+			
					175	T > G	+			

					182	C > -				+
					183	T > G	+			
					185-186	- > G			+	
					191	G > T	+			
					198	G > C	+			
					232	A > G		+		
					234	A > G		+		
					235	A > C	+			
					247	T > A	+			
					248	T > A	+			
					249	T > G	+			
					250	C > G	+			
					251	T > A	+			
					251-252	- > A			+	
					253	T > C		+		
					254	C > G	+			
					269	A > C	+			
					271	A > T	+			
					273	C > -				+
					275	C > G	+			
					292	C > G	+			
					307	T > C		+		
					322	A > G		+		
					323	A > G		+		
					324	G > C	+			
					333	A > C	+			
					337	G > A		+		
					339	T > G	+			
					343	C > G	+			
					346-347	- > C			+	
					346-367	- > G			+	
					350	T > C		+		
					352	G > C	+			
					354	G > C	+			
					357	C > T		+		
					358	A > -				+
					361	G > C	+			
					362	G > -				+
					365	T > G	+			
					372	T > C		+		
					382	A > G		+		
					386	A > T	+			
					388	A > C	+			
					Total		**38**	**16**	**7**	**6**

+ : Negative , - : Positive

Appendix (8).

Type of mutation in the *lasR* gene sequence in *p. aeruginosa* bacteria isolates isolated for Y1, Z3, Z4, Z5, Z6 samples respectively.

NO. of Sample	City	Cause of Burn	Burn Degree	Antibiotic Susceptibility	Site	Point mutation	Type Mutation			
							Transversion	Transition	Insertion	Deletion
Y1	**Najaf**	**Fire**	**Second**	**Sensitive**	46-47	- > T			+	
					46-47	- > G			+	
					48	A > C	+			
					71-72	- > T			+	
					80-81	- > C			+	
					94-95	- > C			+	
					146	T > C		+		
					147	T > G	+			
					153	G > A		+		
					157	G > C	+			
					164	A > G		+		
					173	T > G	+			
					257	T > C		+		
					258	C > A	+			
					259-260	- > G			+	
					292	T > C		+		
					102	A > -				+

					461	C > G	+			
					462	T > C		+		
					477	C > T		+		
					506	T > G	+			
					511	C > G	+			
					521	G > C	+			
					578	C > G	+			
					596	A > C	+			
					605	T > A	+			
					616	C > G	+			
					644	T > G	+			
					647	C > G	+			
					650	T > A	+			
					655-656	- > G			+	
					659	C > T		+		
					662	G > A		+		
					669	A > T	+			
					673	C > A	+			
					675	G > T	+			
					Total		**19**	**9**	**7**	**1**
Y3	**Babel**	**Fire**	**Second**	**Sensitive**	36-37	- > G			+	
					50	C > G	+			
					55	A > C	+			
					81	T > C		+		
					156	T > C		+		
					157	T > G	+			
					159	T > G	+			
					163	G > A		+		
					173	A > G		+		
					174	A > G		+		
					201	A > G		+		
					202	A > T	+			
					203	G > C	+			
					217	C > A	+			
					268-269	- > G			+	
					302	T > C		+		
					312	A > -				+
					334	C > G	+			
					335	C > T		+		
					344	A > C	+			
					370	C > T		+		
					380	G > A		+		
					421	G > C	+			
					426	A > C	+			
					436	C > T		+		
					441	A > G		+		
					453	A > C	+			
					454	C > A	+			
					461	G > T	+			
					466	A > G		+		
					468	T > C		+		
					471	C > G	+			
					472	T > C		+		
					486	A > G		+		
					487	C > T		+		
					496	A > C	+			
					498	C > G	+			
					515	C > G	+			
					516	T > G	+			
					521	C > G	+			
					522	A > G		+		
					526	C > T		+		
					557	C > T		+		
					559	C > G	+			
					561	T > G	+			
					565	C > T		+		
					570	C > G	+			
					588	C > G	+			
					599	C > G	+			
					606	A > C	+			
					611	A > G		+		

					618	A > G		+		
					615	T > A	+			
					616	C > A	+			
					620	C > T		+		
					623	C > G	+			
					624	A > C	+			
					626	C > G	+			
					636	A > G		+		
					643	T > -				+
					649	T > C		+		
					652	A > G		+		
					655	T > G	+			
					658	C > G	+			
					660	A > C	+			
					666-667	- > G			+	
					672	C > A	+			
					673	T > -				+
					675	G > T	+			
					678	C > -				+
					688	A > T	+			
					691	G > T	+			
					693	A > T	+			
					Total		**39**	**27**	**3**	**4**
Y4	**Najaf**	**Fire**	**Third**	**Resistance**	38	A > G		+		
					659	C < G	+			
					Total		**1**	**1**	**0**	**0**
Y5	**Babel**	**liquid**	**Second**	**Resistance**	24-25	- > T			+	
					25	A > G		+		
					32-33	- > A			+	
					38-39	- > A			+	
					39-40	- > A			+	
					41	C > G	+			
					42	C > A	+			
					43-44	- > G			+	
					45	A > C	+			
					49	T > C		+		
					51-52	- > C			+	
					56-57	- > A			+	
					62	T > G	+			
					69	T > C		+		
					94-95	- > C			+	
					95	G > C	+			
					99-100	- > C			+	
					118	A > G		+		
					141	C > G	+			
					143	T > G	+			
					145	T > G	+			
					146	C > A	+			
					147	C > G	+			
					149	G > A		+		
					160	A > G		+		
					203	C > A	+			
					289	T > C		+		
					330	A > C	+			
					412	T > C		+		
					422	C > T		+		
					427	A > G		+		
					440	C > A	+			
					447	G > T	+			
					457	C > G	+			
					458	T > C		+		
					502	T > G	+			
					507	C > G	+			
					534	G > C	+			
					547	T > G	+			
					578	A > C	+			
					592	T > C		+		
					611	T > G	+			
					612	C > G	+			
					637	A > G		+		
					639	T > A	+			
					640	T > G	+			

					642-643	- > G			+	
					646	T > -				+
					652	C > G	+			
					656	C > T		+		
					681	G > A		+		
					Total		**25**	**15**	**10**	**1**
Y6	**Karbala**	**liquid**	**Third**	**Resistance**	27-28	- > T			+	
					28	A > G		+		
					32-33	- > G			+	
					42	C > A	+			
					46	C > G	+			
					152	T > C		+		
					153	T > G	+			
					155	T > G	+			
					159	G > A		+		
					180	T > C		+		
					197	A > G		+		
					198	A > T	+			
					263	T > C		+		
					264	C > A	+			
					264-265	- > G			+	
					277	C > G	+			
					292	T > G	+			
					293	T > C		+		
					298	T > C		+		
					339	A > C	+			
					365	C > T		+		
					367	A > G		+		
					570	C > G	+			
					403	T > G	+			
					407	T > C		+		
					410	G > T	+			
					416	G > C	+			
					436	A > G		+		
					456	G > T	+			
					482	C > T		+		
					494	C > T		+		
					511	T > G	+			
					516	C > G	+			
					552	C > T		+		
					556	T > G	+			
					588	G > C	+			
					595	T > A	+			
					617	C > G	+			
					618	C > -				+
					622	C > G	+			
					650	T > G	+			
					652-653	- > G			+	
					658	T > -				+
					662	C > G	+			
					663	T > C		+		
					664	T > C		+		
					666	C > T		+		
					669	G > A		+		
					672	C > T		+		
					Total		**23**	**20**	**4**	**2**

+ : Negative , - : Positive

Appendix (9).

Type of mutation in the *oprI* gene sequence in *p. aeruginosa* bacteria isolates isolated for D1, D2, D3, D4, D5, D7, D8 samples respectively.

NO. of Sample	City	Cause of Burn	Burn Degree	Antibiotic Susceptibility	Site	Point mutation	Type Mutation			
							Transversion	Transition	Insertion	Deletion
D1	**Najaf**	**Fire**	**Second**	**Resistance**	34-35	- > G			+	
					133	T > C		+		
					124	T > C		+		
					145	T > C		+		
					199	G > T	+			
					Total		**1**	**3**	**1**	

Appendix

D2	**Babel**	**Fire**	**Sensitive**	**Second**	31	C > A	+			
					34-35	- > G			+	
					Total		**1**	**0**	**1**	**0**
D3	**Karbala**	**liquid**	**Sensitive**	**Third**	30	C > A	+			
					33-34	- > G			+	
					35	T > C		+		
					40	T > C		+		
					41	T > A	+			
					43	G > A		+		
					144	T > C		+		
					189	C > T		+		
					191	G > C	+			
					196	C > G	+			
					197	G > C	+			
					198	G > T	+			
					209	C > A	+			
					Total		**7**	**5**	**1**	0
D4	**Blabe**	**Fire**	**Sensitive**	**Second**	35-36	- > G			**+**	
					125	T > C		+		
					Total		**0**	**1**	**1**	**0**
D5	**Babel**	**liquid**	**Resistance**	**Third**	35-36	- > A			+	
					36	C > G	+			
					115	T > C		+		
					126	T > C		+		
					147	T > C		+		
					197	T > A	+			
					201	G > T	+			
					210-211	- > C			+	
					Total		**3**	**3**	**2**	**0**
D7	**Babel**	**liquid**	**Resistance**	**Third**	21	G > C	+			
					35-36	- > G			**+**	
					125	T > C		+		
					Total		**1**	**1**	**1**	**0**
D8	**Babel**	**Fire**	**Sensitive**	**Third**	501-502	- > C			+	
					503	T > A	+			
					509-510	- > C			+	
					510	T > C		+		
					511-512	- > C			+	
					115	T > C		+		
					116	T > C		+		
					527	T > C		+		
					531	G > A		+		
					536	C > A	+			
					538-539	- > C			+	
					541-542	- > C			+	
					542	T > A	+			
					548-549	- > A			+	
					558-559	- > C			+	
					563-564	- > A			+	
					564	C > G	+			
					567	G > A		+		
					570-571	- > A			+	
					574	A > C	+			
					575-576	- > A			+	
					576	T > C		+		
					577	T > C		+		
					585-586	- > C			+	
					585-586	- > C			+	
					587	T > C		+		
					588	T > C		+		
					591-592	- > A			+	
					592	G > A		+		
					595-596	- > C			+	
					600	- > T			+	
					639	C > -				+
					Total		**5**	**11**	**15**	**1**

+ : Negative , - : Positive

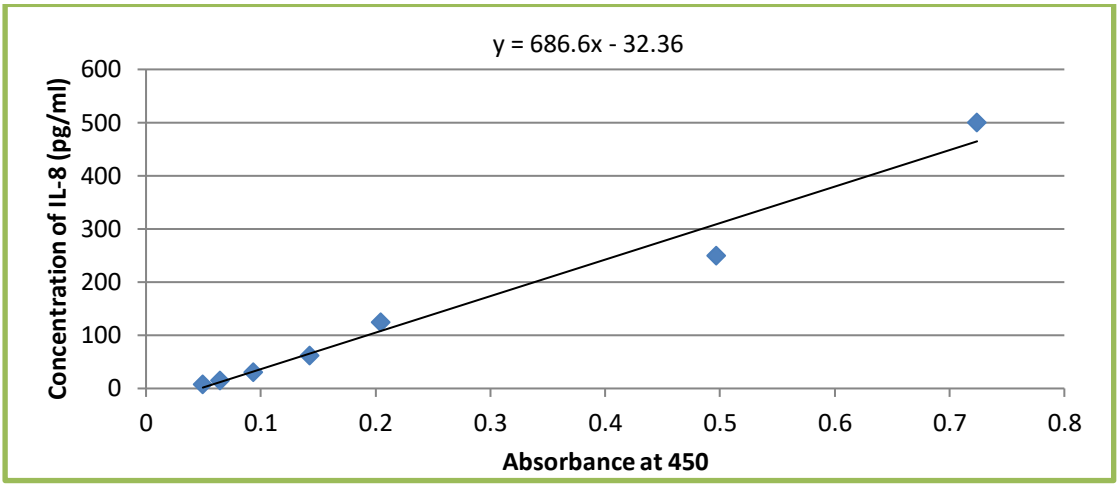
Appendix (10).

Type of mutation in the *oprL* gene sequence in *p. aeruginosa* bacteria isolates isolated for W1, W2, D3, Z4, Z5, Z6, Z8 samples respectively.

NO. of Sample	City	Cause of Burn	Burn Degree	Antibiotic Susceptibility	Site	Point mutation	Type Mutation			
							Transversion	Transition	Insertion	Deletion
W1	Najaf	Fire	Second	Sensitive	10	T > C		+		
					15-16	- > C			+	
					25-26	- > C			+	
					16	A > C	+			
					21	T > C		+		
					78	G > C	+			
					154	G > C	+			
					403	A > -				+
						Total	3	2	2	1
W2	Babel	Fire	Second	Sensitive	15-16	- > C			+	
					16	A > C	+			
					21	A > C	+			
					26	C > G	+			
					41	A > -				+
					156	G > C	+			
					158	G > T	+			
					468	T > C		+		
						Total	5	1	1	1
W3	Karbala	liquid	Third	Sensitive	23	A > C	+			
					27	C > G	+			
					28	C > G	+			
					42	T > -				+
					43	T > -				+
					44	A > C	+			
					83	G > C	+			
					158-159	- > C			+	
					160	G > T	+			
					450	G > -				+
					474	G > -				+
						Total	6	0	1	4
W4	Babel	Fire	Second	Sensitive	10	A > -				+
					156	G > C	+			
						Total	1	0	0	1
W5	Babel	Fire	Second	Resistance	10	G > C	+			
					27-28	- > C			+	
					155	G > C	+			
					164-165	- > A			+	
					166	T > C		+		
					169	G > -				+
						Total	2	1	2	1
W6	Babel	Fire	Third	Sensitive	35	T > -				+
					86	G > -				+
					136	G > A		+		
					159-160	- > C			+	
						Total	0	1	1	2
W8	Babel	Fire	Third	Resistance	26-27	- > C			+	
					362	G > A		+		
					371	T > -				+
					396	T > -				+
					418	G > -				+
					Total		0	1	1	3

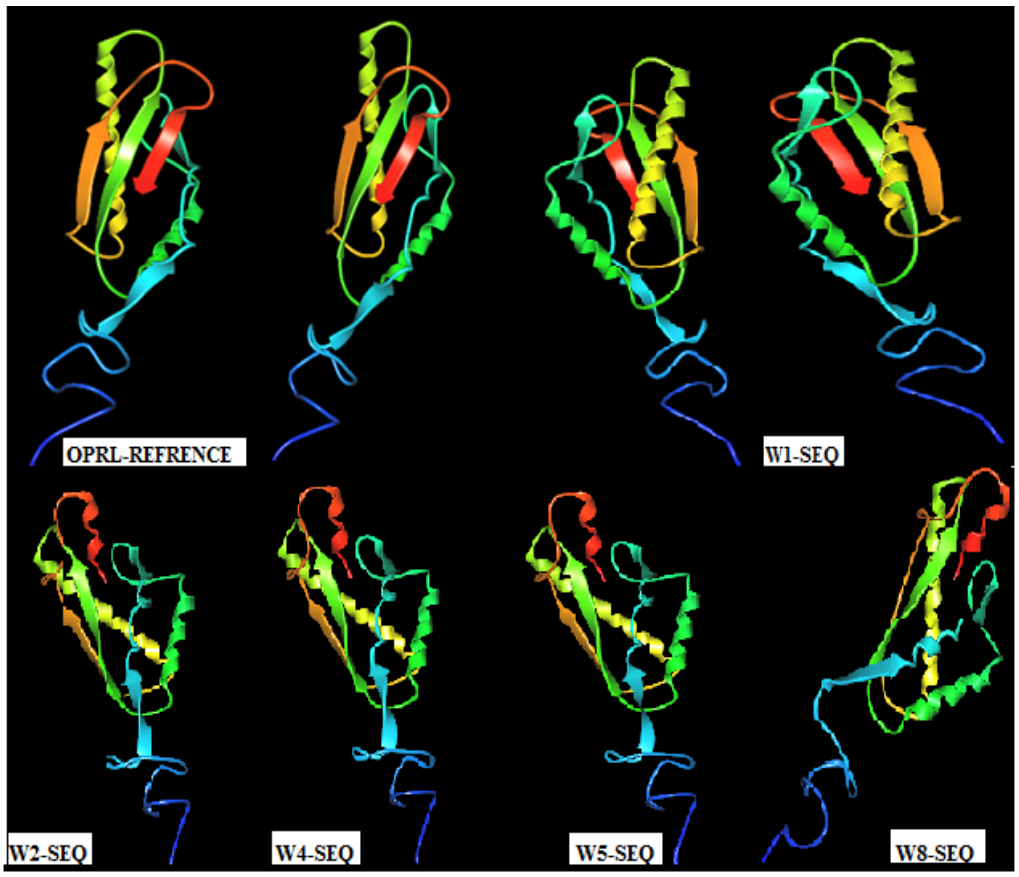
+ : Negative , - : Positive

Appendix (11).



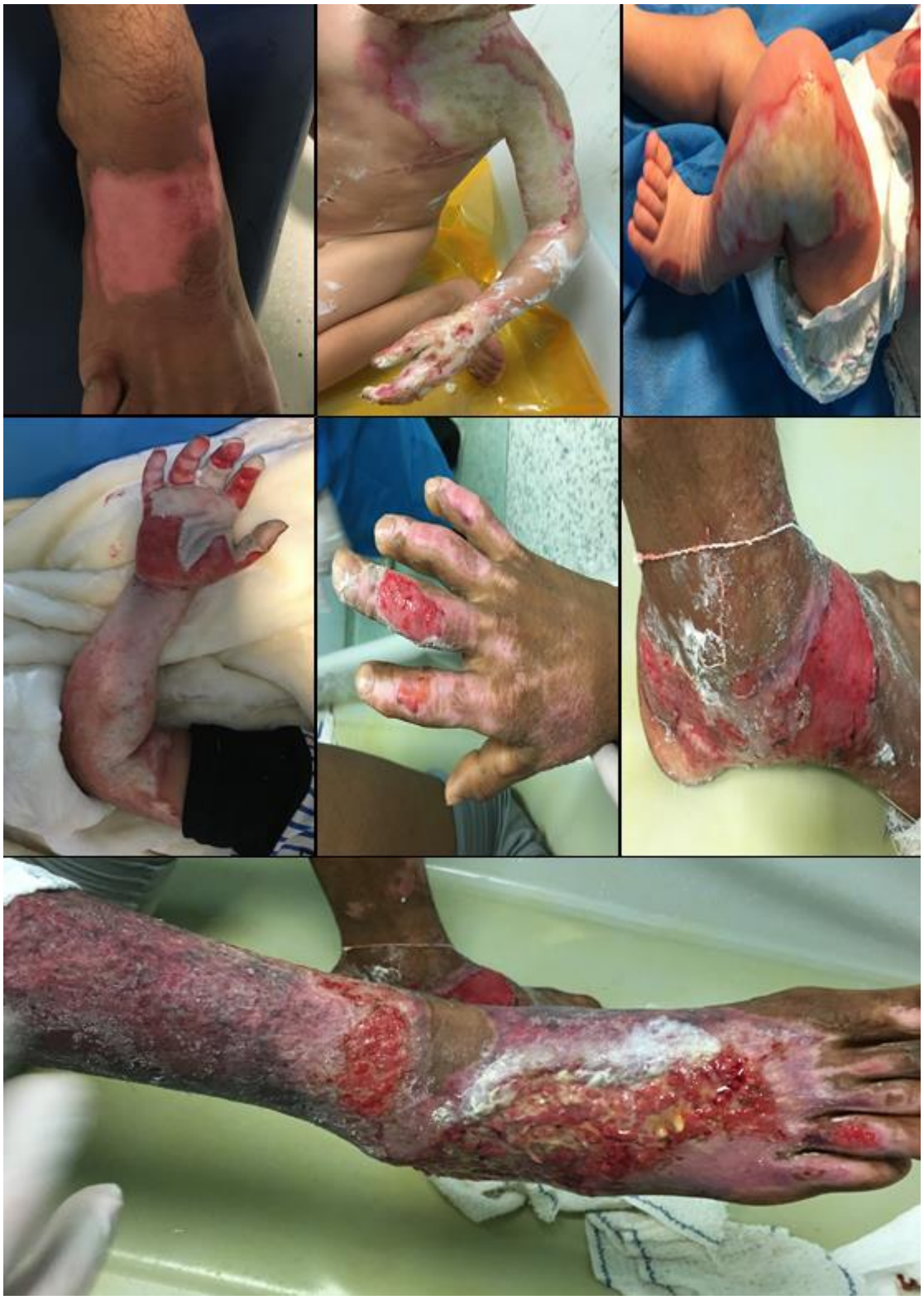
Correlation between the concentration and absorbance of IL-8 in burn patient

Appendix (12).



Three dimensional models of the *P. aeruginosa* for *oprL* proteins.

Appendix (13).



Some burn patients from whom samples were collected

الخلاصة

Pseudomonas aeruginosa هي بكتريا سالبة لصبغة جرام، عصيات هوائية، متحركة بواسطة سوط قطبي, غير مخمرة وليس لها القدرة على تكوين الأبواغ ، بكتريا انتهازية تسبب ارتفاع معدل الإمراضية والوفيات في المرضى الراقدين في المستشفيات الذين يعانون من ضعف في الجهاز المناعي. تمتلك قدرة على اصابة كل انسجة الجسم تقريباً نتيجة لامتلاكها مجموعة كبيرة ومتنوعة من عوامل الضراوة والتي تسهم بشكل كبير في احداث الإمراضية لدى المضيف لذلك فأن الكشف السريع عن هذه البكتريا يلعب دوراً حاسماً في السيطرة على الامراض التي تسببها وخصوصاً في اصابات الحروق .

تم جمع 100 عينة مسحة من مائة مصاب بحروق تم إدخالهم إلى ثلاث مستشفيات مختلفة في ثلاث محافظات ، وحدة الحروق في مستشفى الإمام الصادق (عليه السلام) في بابل ، مركز الحروق في مستشفى الحسين التعليمي بكربلاء , ومركز الحروق في مدينة الصدر الطبية في النجف خلال الفترة من كانون الأول 2020 إلى نيسان 2021. استخدمت عينات المسحات هذه للتحقق من انتشار بكتيريا *P. aeruginosa* ودراسة بعض عوامل الضراوة. بالإضافة إلى ذلك ، تم جمع 40 عينة دم من بعض مرضى الحروق بالإضافة إلى 30 عينة دم من أفراد يتمتعون بصحة جيدة. تم استخدام عينات الدم للتحقق من مستوى المصل من IL-8 بواسطة ELISA.

تمت زراعة عينات المسحات على اكار الدم ، اكار الماكونكي واكار Pseudomonas Chromogenic Agar. تم تأكيد التشخيص واختبار حساسيتها للمضادات الحيوية باستخدام جهاز VITEK2 Compact System. تم اجراء التشخيص الجزيئي بواسطة تقنية PCR ودراسة التسلسل الجيني DNA والتركيب البروتيني الافتراضي لعزلات *P. aeruginosa* باعتماد اربعة جينات: *lasI* و *lasR* و *oprI* و *oprL* .

اظهرت النتائج نسبة حروق الذكور كانت 46% بينما كانت نسبة الاناث 54%, كانت اعلى نسبة اصابة في الاطفال في الفئة العمرية 10-1 سنوات 42% , تليها الفئة العمرية 20-11 بنسبة 27% , واقل نسبة اصابة كانت في الفئة العمرية 60-51 والفئة فوق 61 بنسبة 1%. كان هناك فرق كبير بين الفئات العمرية ($P \leq 0001$) .

اشارت النتائج الى وجود فرق معنوي ($P \leq 0.002$) بين الحالات المشخصة للإصابات من حيث نوع الحروق , حيث كانت اعلى نسبة اصابة بالحروق هي حروق السوائل بنسبة 57% تليها الحرق بواسطة النار بنسبة 41% , وكانت النسبة الاقل من الاصابات لحروق الصدمات الكهربائية بنسبة 2% بالإضافة إلى ذلك ، كانت هناك فروق ذات دلالة إحصائية فيما يتعلق بدرجات معدلات الحروق ، حيث بلغت نسبة حروق الدرجة الثانية 54% وحروق الدرجة الثالثة 45%

اظهرت نتائج الدراسة بأن اكثر انواع البكتريا انتشاراً كانت 67.6% *P. aeruginosa* تليها 12.2% *Klebsiella* , 10.8% *Eschreichia coli* , 4% *Proteus* , واقلها كانت 2.7% *Acinetobacter* و 2.7% *Citrobacter*.

كما اظهرت النتائج ارتفاع معدلات المقاومة لبكتريا *P. aeruginosa* لمعظم المضادات الحيوية وكانت اعلى نسبة مقاومة للمضاد (100%) Cefazolin , يليها (96%) Tigecycline , (88%) Imipenem , (86%) Amikacin , (86%) Levofloxacin , (84%) Gentamicin, (74%) Cefepime , (56%) Ceftazidime , (4%) Ciprofloxacin , بينما اظهرت جميع العينات حساسية عالية (100%) الى Piperacillin/Tazobactem.

فيما يتعلق بالتقنية الجزيئية لعزلات *P .aeruginosa* اظهرت النتائج ان جميع العزلات لديها (100%) جينات *oprL*, *oprI* , و 98% من هذه العزلات لديها جين *lasI* و88 % من العزلات لديها جين *lasR* ضمن الهيكل الوراثي لعزلات بكتريا *P. aeruginosa* قيد الدراسة .

تم اجراء تسلسل المحاذاة لدراسة درجة التشابه مع السلالات العالمية , مقارنة جميع نتائج DNA Sequencing لعزلات *P. aeruginosa* مع قاعدة بيانات NCBI ، وأظهرت نتائج المحاذاة تشابه نسب تسلسل النوكليوتيدات مع نسب القواعد النيتروجينية المخزنة في بنك الجينات إلى نسبة عالية. أظهرت دراسة التركيب البروتيني الافتراضي (insilico) وجود فجوات في النهاية C-terminal وتحول بروتينات β -sheet بسبب بعض التغيرات الوراثية في جينات *lasI* , *lasR* , *oprI* و *oprL*.

بينت نتائج الدراسة الحالية أن تركيز الانترلوكين-8 في مجموعة مرضى الحروق ادى الى زيادة معنوية في مستوى مصل مرضى الدرجتين الثانية والثالثة ($P \leq 0.001$) وكانت هناك زيادة معنوية في مستوى الانترلوكين-8 بين مرضى العدوى البكتيرية في مقارنة مع مسحات النمو السلبية في نفس درجة الحرق عند ($P \leq 0.05$) .

توصلت هذه الدراسة إلى ان انتشار *P. aeruginosa* مرتفع بين العينات السريرية و وجد أن عزلات *P. aeruginosa* مقاومة لمعظم المضادات الحيوية باستثناء Piperacillin/Tazobactem التي كانت حساسة له (100%). وجود جينات *lasI* , *lasR* , *oprI* و *oprL* بين عزلات *P. aeruginosa* ربما يعتبر بمثابة طرق بديلة لتشخيص هذه البكتيريا بالطرق الجزيئية القائمة على تقنية تفاعل PCR و DNA Sequencing . علاوة على ذلك ، استخدام الانترلوكين-8 كمؤشر حيوي للعمليات الالتهابية التي تسببها الحروق ، خاصة بين المرضى المصابين بعدوى بكتيرية بنفس الدرجة.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

كلية العلوم الطبية التطبيقية / جامعة كربلاء

قسم التحليلات المرضية

دراسة جنائية لبعض المتغيرات المناعية والبكتريولوجية بين ضحايا الحروق

رسالة مقدمة

الى مجلس كلية العلوم الطبية التطبيقية - جامعة كربلاء

وهي جزء من متطلبات نيل شهادة الماجستير في التحليلات المرضية - علوم الادلة الجنائية

مقدمة من قبل

حيدر نعمة عوده الجبوري

بكالوريوس / كلية العلوم

جامعة بابل (2008)

بأشراف

الاستاذ الدكتور	الاستاذ المساعد
علي حمود السعدي	اسراء سعيد عباس

1443هـ

2021 م